CARDIOVASCULAR CONTROL DURING EXERCISE AND THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN HEART FAILURE WITH REDUCED EJECTION FRACTION

by

Zachary Barrett-O'Keefe

A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Exercise and Sport Science

The University of Utah

August 2015

The University of Utah Graduate School

STATEMENT OF DISSERTATION APPROVAL

The dissertation of **Zachary Barrett-O'Keefe M.S.**

has been approved by the following supervisory committee members:

D. Walter Wray	, Chair	**10/31/2014** Date Approved
Russell S. Richardson	, Member	**10/31/2014** Date Approved
J. David Symons	, Member	**10/31/2014** Date Approved
Markus M. Amann	, Member	**10/31/2014** Date Approved
John R. Halliwill	, Member	**10/31/2014** Date Approved

and by **Janet M. Shaw**, Chair/Dean of

the Department/College/School of **Exercise and Sport Science**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

The objective of this dissertation was to systematically investigate the hemodynamic response to exercise in heart failure with reduced ejection fraction (HFrEF) and healthy individuals of a similar age, with an emphasis on how the sympathetic nervous system (SNS) may contribute to the dysregulation of the cardiovascular system in this cohort. The first study aimed to determine how varying levels of metaboreceptor activation alters the mean arterial pressure (MAP) response as well as the degree in which cardiac output (CO) and systemic vascular conductance (SVC) contribute to the metaboreflex-induced increase in MAP. We observed similar increases in MAP induced by metaboreceptor activation in both groups; however, this response was driven primarily by increases in CO in the control group and reductions in SVC in the HFrEF group. These data suggest a preserved role of the metaboreflex-induced increase in MAP in HFrEF, but suggest that this response is governed by the peripheral circulation in this cohort, a maladaptation that may exacerbate systolic dysfunction through an increase in afterload. The second study of this dissertation was focused on investigating the peripheral vasodilatory and hyperemic response to exercise in isolation of central hemodynamic limitations in both the upper and lower limbs. This study documented an impaired hyperemic response to both static-intermittent handgrip exercise as well as dynamic single-leg knee-extensor exercise in HFrEF patients - impairments primarily attributed to vasodilatory dysfunction, as the increase in MAP

induced by these exercise modalities was preserved compared to healthy individuals. Together, these findings have identified a significant attenuation of the exercise-induced hyperemic response during both upper and lower limb exercise, implicating maladaptions in the peripheral hemodynamic response to exercise as a potential contributor limiting exercise capacity in this patient group. The third study sought to address the contribution of the alpha-adrenergic receptor pathway in the regulation of blood flow to exercising skeletal muscle in HFrEF patients. At rest, alpha-1-adrenergic receptor vasoconstriction induced by local intra-arterial infusion of phenylephrine (PE) was reduced in HFrEF compared to control subjects. During exercise, the vasoconstrictor responsiveness to PE was significantly attenuated in the control group and preserved in HFrEF patients compared to rest. Additionally, nonspecific alpha-adrenergic receptor antagonism induced by local intra-arterial infusion of phentolamine increased blood flow to a greater degree in HFrEF compared to the control subjects, both at rest and during exercise. Together, these findings demonstrate a marked contribution of alpha-adrenergic receptor restraint of leg blood flow in HFrEF patients during exercise. Collectively, these three studies have provided new insight into the role the SNS and peripheral hemodynamics play in the maladaptive cardiovascular response to exercise displayed in patients with HFrEF, further implicating the peripheral expression of SNS activity as a primary contributor to impaired exercise capacity in this patient group.

TABLE OF CONTENTS

LIST OF FIGURES

Figure

LIST OF TABLES

Table

ACKNOWLEDGMENTS

There are countless people to thank for their support and encouragement during the time it took me to complete this dissertation. I would like to take this opportunity to thank a few of these people who helped make the completion of this dissertation possible.

I wish to express my sincere appreciation to my advisor, Dr. Wray, for bringing me to the University of Utah, sharing with me your knowledge, vision, and enthusiasm for physiology, as well as giving me the intellectual freedom to grow under your advising. This dissertation would not have been possible without your guidance and support. To my fortune, I have also benefitted from the advising of Dr. Richardson, Dr. Amann, Dr. Symons, and Dr. Halliwill. I am grateful for the time, effort, and unique perspective that you each brought to this endeavor.

To the members of the Utah Vascular Research Laboratory (UVRL): thank you for sacrificing your time and energy to help me develop and complete the research studies comprising this dissertation. Additionally, work we do at the UVRL would not be possible without the subjects who volunteer their time, so I would like to thank all of the subjects who participated in my studies over the years.

To Dr. Halliwill and the graduate students I had the privilege and honor of working with during my undergraduate years in the Department of Human Physiology at the University of Oregon: thank you for instilling in me the love for the pursuit of knowledge.

Finally, I would like to dedicate this dissertation to my family. Throughout the years, your unconditional love and words of wisdom have helped keep me grounded and maintain a positive outlook on life.

CHAPTER 1

INTRODUCTION

In the United States, chronic heart failure (CHF) constitutes one of the leading causes of mortality, accounting for roughly one third of all deaths from cardiovascular causes with a 50% 5-year mortality rate (52). Additionally, the American Heart Association estimates that more than 5 million people in the United States alone have CHF, with roughly 550,000 new cases diagnosed each year (52). CHF is a disease that has many different origins and is typically the most severe manifestation of almost every form of cardiac disease, including myocardial infarction, coronary atherosclerosis, hypertension, congenital heart disease, and cardiomyopathies (27).

CHF is a pathology which is commonly characterized by left ventricular ejection fraction (LVEF), an expression of stroke volume (SV) as a percentage of left ventricular end-diastolic volume. While the crudity of LVEF as a measure of left ventricular function has been criticized, the association between LVEF and morbidity as well as mortality is well established (8, 67). However, CHF is a subjective clinical syndrome which is variably defined (34), and, in general, heart failure classification is poorly related to LVEF (34). For example, patients with a reduced LVEF may be asymptomatic, while severe CHF-related symptoms may be apparent in patients with a “preserved” LVEF. Thus, patients with CHF are now further delineated on the basis of LVEF, leading to the new nomenclature of heart failure with reduced ejection fraction (HFrEF) or heart failure with preserved ejection fraction (HFpEF) (29).

Autonomic Dysfunction in Heart Failure with Reduced Ejection Fraction

A hallmark characteristic of patients with HFrEF is heightened sympathetic nervous system (SNS) activity, quantified by resting muscle sympathetic nerve activity

(MSNA) (16, 32) and plasma norepinephrine (NE) concentration (17), both of which are associated with severity (16, 17) and predictive of mortality in HFrEF patients (3, 17). Additionally, observations from clinical trials have indicated that exaggerated SNS activation appears to precede the development of clinical signs and symptoms of HFrEF (17), suggesting that sympathoexcitation plays a role in the genesis and progression of the disease. While this increased SNS activity initially serves to improve cardiac function and support arterial blood pressure (ABP), it also provokes a sustained peripheral vasoconstriction that restrains skeletal muscle blood flow (63, 81). For this reason, the evolving model of pathophysiology in CHF has expanded to include the peripheral vasculature, where the disease manifests as vascular and skeletal muscle dysfunction related to underlying autonomic effects of the disease.

One of the principal functional consequences of the exaggerated SNS activation and subsequent peripheral vasoconstriction in patients with HFrEF might be the worsening of symptoms upon exertion. Indeed, HFrEF patients have been documented to have severe exercise intolerance which is associated with the severity of the disease and triggers debilitating cases of dyspnea and fatigue (63). Interestingly, while central hemodynamic abnormalities are, by definition, the fundamental characterization of HFrEF, the degree of left ventricular dysfunction does not fully explain the degree of exercise intolerance or symptom status in this patient group (4, 22, 36, 65, 68). This supports the possibility that peripheral maladaptations may contribute to the exercise intolerance in this patient group. Early studies exploring the possible relation between exercise intolerance in HFrEF and SNS activity indicated that resting MSNA is negatively associated with aerobic capacity in this cohort (42), and patients with HFrEF

exhibit an exaggerated increase in MSNA during exercise (37, 43, 60, 62). These novel findings raise the question of the functional end-organ consequences of the persistent augmentation of MSNA during exercise in HFrEF.

The Metaboreflex Response to Exercise in Heart Failure with Reduced Ejection Fraction

As mentioned above, accumulating evidence indicates that exercise limitations in HFrEF patients are not predominantly due to inadequate left ventricular function, which has led to a shift in the investigations probing the mechanisms contributing to exercise intolerance in this patient population to the periphery. Recently, Drs. Coats and Piepoli have hypothesized that abnormalities arising from the skeletal muscle, specifically the sensory nerve fibers that mediate reflex changes triggered by exercise, may contribute to the exercise limitations in HFrEF (6, 48). This so-called “muscle hypothesis” of HF proposes another cycle of deterioration similar to that of neuroendocrine activation, whereby left ventricular dysfunction leads to abnormalities in skeletal muscle metabolism and function that contributes to an exaggerated reflex increase in SNS activity.

Located within the skeletal muscle, the sensory afferent fibers mediating these reflex increases in SNS activity, often termed the “ergoreflex”, consist of group III afferent fibers which are predominately mechanically sensitive (mechanoreceptors) and group IV afferent fibers (metaboreceptors) which are principally sensitive to metabolites produced during exercise (26). These afferent signals are carried to the cardiovascular center of the nucleus tractus solitarii (NTS), provoking specific efferent signals to the heart and peripheral circulation. Specifically, vagal withdrawal and an increase in SNS activity to the heart induces an increase in heart rate, which is accompanied by a

sympathetically-mediated increase in SV (1, 7, 44), collectively increasing the perfusion pressure generated by the heart. In the periphery, SNS activation helps divert blood flow to the exercising skeletal muscle through vasoconstriction of the vasculature perfusing less metabolically active tissue (2, 38, 39). Together, these cardiac and vascular responses work in concert to increase perfusion of the muscle from which the afferent signals emanate (1, 26, 45).

The extent to which the mechano- and metaboreceptors contribute to the reflex increase in SNS activity (as measured by direct recordings of MSNA) in HFrEF patients has long been a topic of debate (40, 49). Implementing postexercise circulatory occlusion following handgrip exercise, previous studies have documented an increased sensitivity of both the mechanoreflex (37) and the metaboreflex (43, 60). However, conflicting evidence exists in regards to the metaboreflex, with studies indicating both a blunted (62) and a similar (37) increase in MSNA in HFrEF patients compared to healthy individuals. Under a closer lens, it appears that these conflicting results can be attributed to the modality of handgrip exercise and the intensity of exercise implemented. Indeed, Sterns *et al.* (62) had subjects perform static handgrip exercise to activate the metaboreflex, an exercise modality that is not rhythmic in nature, making it difficult to translate these finding to physical activity performed by HFrEF patients in everyday life. Furthermore, the intensity of rhythmic handgrip exercise implemented by Middlekauff *et al.* (37) failed to significantly increase MSNA during metaboreceptor activation in either HFrEF patients or healthy individuals, making it difficult to conclude that there is no difference in the metaboreflex control of MSNA between groups. Based on the conflicting findings on the role of the metaboreflex in increasing MSNA during physical stress in HFrEF, it is

not surprising that studies investigating the impact of this increase in SNS activity on ABP responses have been inconclusive as well. Indeed, studies have documented both exaggerated (48, 57) and similar (5, 30, 43, 62) increases in ABP during metaboreceptor activation in HFrEF patients compared to healthy individuals. The conflicting findings from these studies indicate that significant uncertainty still remains as to whether the metaboreflex-induced pressor response is altered in HFrEF.

Additionally, very little is known about the roles cardiac output (CO) and systemic vascular conductance (SVC) play in increasing ABP during metaboreceptor activation in HFrEF. In a healthy animal model, O'Leary *et al.* (44) documented that the rise in ABP triggered by metaboreceptor activation was solely due to increases in CO during low to moderate exercise intensities. Additionally, during near maximal exercise, where increases in CO are minimal, Augustyniak *et al*. (2) documented that a further increase in ABP due to metaboreceptor activation was due to a reduction in SVC. This is in contrast to findings in an animal model of HF, where reductions in SVC predominately contribute to the metaboreflex-induced increase in ABP during all exercise intensities, a response likely due to impaired left ventricular function (19). In humans, even less is known about the degree in which CO and SVC contribute to the metaboreflex-induced increase in ABP in HFrEF. In one of the only studies exploring this topic, Crisafulli *et al*. (7) utilized one moderate intensity of rhythmic handgrip exercise to study this relationship in HFrEF and healthy controls, and observed a metaboreflex-induced increase in ABP which was predominantly driven by an increase in CO in healthy individuals and a reduction in SVC in patients with HFrEF. This has been complemented by work indicating an exaggerated increase in diastolic blood pressure during

metaboreceptor activation in HFrEF, indirectly indicating a reduction in SVC in this patient group (47). While these investigations have shed light on the differing avenues by which the metaboreflex increases ABP in HFrEF compared to their healthy counterparts, the inclusion of only one moderate intensity workload of handgrip exercise leaves unanswered whether the relative contribution of CO and SVC to the exercise pressor response varies as a consequence of exercise intensity.

A more comprehensive understanding of the metaboreflex, and the relative contribution of CO and SVC to this reflex response, would add significantly to our understanding of the "muscle hypothesis" in HFrEF. Undeniably, an attenuation in the rise in CO coupled with an exaggerated reduction in SVC may collectively generate a scenario where blood flow to exercising skeletal muscle is potentially compromised due to central and peripheral hemodynamic alterations in order to preserve ABP. Indeed, reductions in SVC, a measure of systemic vascular tone which also represents the nonpulsatile component of arterial afterload (25, 80), may present a further stress to cardiac muscle, a stress which is likely vastly minimized in healthy individuals. It follows that knowing whether reductions in SVC play a primary role in the metaboreflex-induced increases in ABP across a wide array of exercise intensities will indicate at what level of work this reflex pathway might become detrimental in increasing perfusion of the exercising skeletal muscle. This concept was investigated in Specific Aim 1 of this dissertation.

Peripheral Hemodynamic Response to Exercise in Heart Failure with Reduced Ejection Fraction

While an exaggerated metaboreflex response to exercise may contribute importantly to exercise intolerance in HFrEF, these patients may also exhibit a fundamental impairment in vasodilatory ability and hyperemia during physical activity. Initial evidence for a functional role of impaired peripheral hemodynamics in limiting exercise capacity in this cohort was documented during peak cycling exercise, where an apparent reduction in leg vascular conductance was observed in patients with HFrEF compared to healthy individuals (71-75). However, the use of a large muscle mass exercise paradigm presents limitations in distinguishing abnormalities in peripheral vasomotor function in HFrEF. When performing exercise, blood flow to exercising skeletal muscle is accomplished through an orchestrated series of events which includes contributions from both "central" (i.e. CO) and peripheral (i.e. SVC) portions of the cardiovascular system in order to divert blood away from less metabolically active tissue towards exercising skeletal muscle. During exercise which recruits a large fraction of total body muscle mass, central circulatory factors play an increasingly important role in the preservation of ABP (46, 51, 55, 56). Due to impaired left ventricular function, patients with HFrEF cannot increase CO to the same level as healthy individuals during exercise (63, 75). However, irrespective of cardiac function, there is no difference in the observed MAP during peak cycling exercise in HFrEF compared to healthy controls (63). This may indicate that a restraint of the exercise-induced increase in SVC plays a heightened role in ABP regulation in HFrEF, and may also indicate that the observed reduction in leg vascular conductance and associated reduction in perfusion of the

exercising skeletal muscle might solely be due to the maintenance of ABP versus limitations in vasodilatory capacity in this patient group (63, 75).

In a small number of studies, the utilization of small muscle mass exercise such as dynamic single-leg knee-extensor (KE) and rhythmic handgrip exercise has allowed for a more thorough investigation of peripheral vasomotor responses to exercise in HFrEF in isolation of the significant cardiac stress and associated confounding effects of ABP regulation imposed by large muscle mass exercise. The results, however, have been equivocal. During handgrip exercise, patients with HFrEF have been documented to possess both similar (58) as well as attenuated (23, 81) vasodilatory and hyperemic responses in the exercising limb, differences which have been attributed to lack of standardization with respect to exercise cadence and intensity (35, 58, 69, 76, 81). In studies which utilized the single-leg KE exercise model, similar increases in leg vascular conductance and leg blood flow have been reported between HFrEF and healthy individuals across a range of exercise intensities (15, 33). However, since these studies were performed, the clinical treatment of HFrEF has evolved, with the inclusion of new pharmacological interventions (18, 21, 31, 66) which might indirectly alter vasomotor regulation in response to small muscle mass exercise. Understanding the peripheral vasomotor response to small muscle mass exercise in the presence of these recently implemented pharmacological interventions could enlighten clinicians on how the progression of clinical treatment of patients with HFrEF has affected vascular regulation during exercise. Thus, the objective of Specific Aim 2 of this dissertation was to utilize both arm and leg exercise models to provide a more definitive and comprehensive

assessment of the limb vasodilatory and hyperemic response during small muscle mass exercise in HFrEF patients on modern, optimized pharmacotherapy.

Alpha-Adrenergic Receptor Control of Skeletal Muscle Blood Flow during Exercise in Heart Failure with Reduced Ejection Fraction

In the periphery, MSNA is expressed though the stimulation of alpha-adrenergic receptors, located on the vascular smooth muscle, leading to vasoconstriction. A small number of prospective studies using acute alpha-adrenergic receptor agonist and antagonist drug administration have provided some insight concerning the expression of sympathoexcitation at the end organ in HFrEF. Zelis *et al.* (81) reported a qualitatively greater percent increase in forearm blood flow between HFrEF and control groups following intra-arterial infusion of phentolamine (PHEN), a nonselective alpha-adrenergic receptor antagonist. Similarly, brachial artery infusion of phenylephrine (PE) and BHT-933 (selective alpha-1 and alpha-2 agonists, respectively) in HFrEF patients and unmatched, healthy controls produced equivalent decreases in forearm blood flow, despite a substantially higher plasma NE concentration in the HFrEF patients (24). Findings from these previous studies in HFrEF are somewhat difficult to reconcile with more recent work in other populations with elevated SNS activity, where a downregulation and/or desensitization of alpha-adrenergic receptors is observed in response to chronic sympathoexcitation. For example, in the elderly, a population in which SNS activity is also elevated (10, 41, 64), Dinenno *et al.* (14) observed a substantially larger increase in leg blood flow to intra-arterial infusion of PHEN in healthy, older individuals compared to their younger counterparts. Our group (79) and

others (9, 61) have observed a blunted alpha-adrenergic receptor vasoconstriction in response to intra-arterial sympathomimetic infusions, which likely represents a protective response to the 200-300% increase in resting SNS activity and augmented endogenous contribution of the alpha-adrenergic receptors to vascular tone reported with advancing age.

This lack of adaptation to high SNS activity in HFrEF likely predisposes these patients to an exaggerated cardiovascular response to acute sympathoexcitation, such as is seen during physical activity. Indeed, in healthy individuals, vascular conductance in the vessels perfusing exercising skeletal muscle is optimized in large part by exercise-induced reductions in alpha-adrenergic receptor sensitivity, a phenomenon termed "functional sympatholysis" (50). However, in HFrEF, the disease-related increase in SNS activity and sensitivity of alpha-adrenergic receptors may produce a level of vasoconstriction in the exercising muscle vasculature that cannot be entirely overcome, resulting in a sustained reduction in vascular conductance and blood flow in this cohort. Though a growing number of studies from our group (77-79) and others (11-13, 20, 28, 53, 54, 59, 70) have examined sympatholysis in healthy humans, very little is known concerning whether sympathetic vasoconstriction is altered in HF. In humans, early work performed by Zelis *et al.* (81) identified a substantial reduction in blood flow during rhythmic handgrip exercise in HFrEF patients, suggesting an "inadequate arteriolar vasodilation" that was partially restored when the exercise was repeated in the presence of intra-arterial infusion of PHEN. However, this response was not evident in a subsequent study using a similar pharmacologic approach during maximal upright cycling (72), and thus the evidence implicating sympathetic overactivity and subsequent

vasoconstriction of the exercising muscle vasculature of HFrEF patients remains equivocal.

To our knowledge, no previous studies have directly tested whether sympatholysis is impaired in HFrEF patients, or determined if the observations concerning arm alpha-adrenergic receptor responses can be extended to the vasculature of large, locomotory muscle groups. Specific Aim 3 of this dissertation thus sought to close this important knowledge gap by examining the role the alpha-adrenergic receptor pathway plays in the regulation of peripheral vasomotor tone and blood flow at rest and during exercise in HFrEF patients.

Hypotheses and Specific Aims

The objective of this dissertation was to systematically examine the hemodynamic response to exercise in HFrEF and healthy control subjects of a similar age, and to determine the contribution of the metaboreflex and the alpha-adrenergic receptor pathway in the regulation of blood flow and vascular conductance in this cohort. Specifically, the first study determined how varying levels of metaboreceptor activation alters the ABP response in HFrEF patients, and established the degree to which CO and SVC contribute to the metaboreflex-induced pressor response. The second study utilized small muscle mass exercise in patients with HFrEF to further investigate the limb-specific peripheral vasodilatory and hyperemic response to exercise in isolation of central hemodynamic limitations. Building on these studies, the third study examined the contribution of the alpha-adrenergic receptor pathway as a mechanism responsible for the disease-related impairment in exercising muscle blood flow in HFrEF patients.

Specific Aim 1

Further elucidate the ABP response to varying levels of metaboreceptor activation and determine the relative contribution of CO and SVC to the metaboreflex response in HFrEF and healthy, age-matched controls.

Hypothesis 1

HFrEF patients would exhibit similar increases in MAP across all levels of metaboreceptor activation compared to healthy controls.

Hypothesis 2

HFrEF patients would exhibit a greater dependence on reductions in SVC than increases in CO to achieve the metaboreflex-induced pressor response compared to healthy controls.

Hypothesis 3

HFrEF patients would exhibit greater increases in arterial afterload and attenuated increases in functional systolic work in response to metaboreceptor activation compared to healthy controls.

Specific Aim 2

Investigate limb-specific peripheral hemodynamic responses to exercise in isolation of central hemodynamic limitations in patients with HFrEF and healthy controls.

Hypothesis 1

During both static-intermittent handgrip and single-leg KE exercise, patients with HFrEF would exhibit an attenuated hyperemic response driven by an impaired vasodilatory capacity compared to healthy controls.

Specific Aim 3

Determine the degree to which chronically elevated SNA is expressed in the peripheral circulation, both at rest and during dynamic exercise, in patients with HFrEF and healthy, age-matched controls.

Hypothesis 1

a) At rest, vasoconstriction in response to alpha-1-adrenergic receptor agonist drug infusion (PE) would reduce leg blood flow to a similar degree in HFrEF patients compared to healthy, age-matched controls.

b) At rest, a greater vasodilation in response to alpha-adrenergic receptor antagonist drug infusion (PHEN) would contribute to an enhanced hyperemic response in HFrEF patients compared to healthy, age-matched controls.

Hypothesis 2

a) During exercise, HFrEF patients would exhibit a sustained responsiveness to alpha-1-adrenergic receptor agonist drug infusions into the exercising limb, such that functional sympatholysis would be reduced in HFrEF patients compared to healthy, age-matched controls.

b) During exercise, HFrEF patients would experience a greater vasodilation to alpha-adrenergic receptor antagonist drug infusion in the exercising limb, such that

sympathetic restraint of exercising limb blood flow would be greater in HFrEF patients compared to healthy, age-matched controls.

References

1. **Amann M, Runnels S, Morgan DE, Trinity JD, Fjeldstad AS, Wray DW, Reese VR, and Richardson RS**. On the contribution of group III and IV muscle afferents to the circulatory response to rhythmic exercise in humans. *J Physiol* 589: 3855-3866, 2011.

2. **Augustyniak RA, Collins HL, Ansorge EJ, Rossi NF, and O'Leary DS**. Severe exercise alters the strength and mechanisms of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol* 280: H1645-1652, 2001.

3. **Barretto AC, Santos AC, Munhoz R, Rondon MU, Franco FG, Trombetta IC, Roveda F, de Matos LN, Braga AM, Middlekauff HR, and Negrao CE**. Increased muscle sympathetic nerve activity predicts mortality in heart failure patients. *Int J Cardiol* 135: 302-307, 2009.

4. **Benge W, Litchfield RL, and Marcus ML**. Exercise capacity in patients with severe left ventricular dysfunction. *Circulation* 61: 955-959, 1980.

5. **Carrington CA, Fisher WJ, Davies MK, and White MJ**. Muscle afferent and central command contributions to the cardiovascular response to isometric exercise of postural muscle in patients with mild chronic heart failure. *Clin Sci (Lond)* 100: 643-651, 2001.

6. **Coats AJ, Clark AL, Piepoli M, Volterrani M, and Poole-Wilson PA**. Symptoms and quality of life in heart failure: the muscle hypothesis. *Br Heart J* 72: S36-39, 1994.

7. **Crisafulli A, Salis E, Tocco F, Melis F, Milia R, Pittau G, Caria MA, Solinas R, Meloni L, Pagliaro P, and Concu A**. Impaired central hemodynamic response and exaggerated vasoconstriction during muscle metaboreflex activation in heart failure patients. *Am J Physiol Heart Circ Physiol* 292: H2988-2996, 2007.

8. **Curtis JP, Sokol SI, Wang Y, Rathore SS, Ko DT, Jadbabaie F, Portnay EL, Marshalko SJ, Radford MJ, and Krumholz HM**. The association of left ventricular ejection fraction, mortality, and cause of death in stable outpatients with heart failure. *J Am Coll Cardiol* 42: 736-742, 2003.

9. **Dinenno FA, Dietz NM, and Joyner MJ**. Aging and forearm postjunctional alpha-adrenergic vasoconstriction in healthy men. *Circulation* 106: 1349-1354, 2002.

10. **Dinenno FA, Jones PP, Seals DR, and Tanaka H**. Limb blood flow and vascular conductance are reduced with age in healthy humans: relation to elevations in sympathetic nerve activity and declines in oxygen demand. *Circulation* 100: 164-170, 1999.

11. **Dinenno FA, and Joyner MJ**. Blunted sympathetic vasoconstriction in contracting skeletal muscle of healthy humans: is nitric oxide obligatory? *J Physiol* 553: 281-292, 2003.

12. **Dinenno FA, and Joyner MJ**. Combined NO and PG inhibition augments alpha-adrenergic vasoconstriction in contracting human skeletal muscle. *Am J Physiol Heart Circ Physiol* 287: H2576-2584, 2004.

13. **Dinenno FA, Masuki S, and Joyner MJ**. Impaired modulation of sympathetic alpha-adrenergic vasoconstriction in contracting forearm muscle of ageing men. *J Physiol* 567: 311-321, 2005.

14. **Dinenno FA, Tanaka H, Stauffer BL, and Seals DR**. Reductions in basal limb blood flow and vascular conductance with human ageing: role for augmented alpha-adrenergic vasoconstriction. *J Physiol* 536: 977-983, 2001.

15. **Esposito F, Reese V, Shabetai R, Wagner PD, and Richardson RS**. Isolated quadriceps training increases maximal exercise capacity in chronic heart failure: the role of skeletal muscle convective and diffusive oxygen transport. *J Am Coll Cardiol* 58: 1353-1362, 2011.

16. **Ferguson DW, Berg WJ, and Sanders JS**. Clinical and hemodynamic correlates of sympathetic nerve activity in normal humans and patients with heart failure: evidence from direct microneurographic recordings. *J Am Coll Cardiol* 16: 1125-1134, 1990.

17. **Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH, Rudin-Toretsky E, and Yusuf S**. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation* 82: 1724-1729, 1990.

18. **Gottlieb SS, McCarter RJ, and Vogel RA**. Effect of beta-blockade on mortality among high-risk and low-risk patients after myocardial infarction. *N Engl J Med* 339: 489-497, 1998.

19. **Hammond RL, Augustyniak RA, Rossi NF, Churchill PC, Lapanowski K, and O'Leary DS**. Heart failure alters the strength and mechanisms of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol* 278: H818-828, 2000.

20. **Hansen J, Sander M, and Thomas GD**. Metabolic modulation of sympathetic vasoconstriction in exercising skeletal muscle. *Acta Physiol Scand* 168: 489-503, 2000.

21. **Heidenreich PA, Lee TT, and Massie BM**. Effect of beta-blockade on mortality in patients with heart failure: a meta-analysis of randomized clinical trials. *J Am Coll Cardiol* 30: 27-34, 1997.

22. **Higginbotham MB, Morris KG, Conn EH, Coleman RE, and Cobb FR**. Determinants of variable exercise performance among patients with severe left ventricular dysfunction. *Am J Cardiol* 51: 52-60, 1983.

23. **Imaizumi T, Hirooka Y, and Takeshita A**. Decreased skeletal muscle vasodilation in patients with congestive heart failure. *Jpn Circ J* 56: 500-503, 1992.

24. **Indolfi C, Maione A, Volpe M, Rapacciuolo A, Esposito G, Ceravolo R, Rendina V, Condorelli M, and Chiariello M**. Forearm vascular responsiveness to alpha 1- and alpha 2-adrenoceptor stimulation in patients with congestive heart failure. *Circulation* 90: 17-22, 1994.

25. **Kass DA, and Kelly RP**. Ventriculo-arterial coupling: concepts, assumptions, and applications. *Annals of biomedical engineering* 20: 41-62, 1992.

26. **Kaufman MP, and Hayes SG**. The exercise pressor reflex. *Clin Auton Res* 12: 429-439, 2002.

27. **Kemp CD, and Conte JV**. The pathophysiology of heart failure. *Cardiovasc Pathol* 21: 365-371, 2012.

28. **Kirby BS, Markwald RR, Smith EG, and Dinenno FA**. Mechanical effects of muscle contraction do not blunt sympathetic vasoconstriction in humans. *Am J Physiol Heart Circ Physiol* 289: H1610-1617, 2005.

29. **Koker IH, Pamuk ON, Karlikaya C, Tuncbilek N, and Cakir N**. A low prevalance of purified protein derivative test positivity in Turkish patients with rheumatoid arthritis. Association with clinical features and HRCT findings. *Clin Exp Rheumatol* 25: 54-59, 2007.

30. **Kon H, Nakamura M, Arakawa N, and Hiramori K**. Muscle metaboreflex is blunted with reduced vascular resistance response of nonexercised limb in patients with chronic heart failure. *J Card Fail* 10: 503-510, 2004.

31. **Lechat P, Packer M, Chalon S, Cucherat M, Arab T, and Boissel JP**. Clinical effects of beta-adrenergic blockade in chronic heart failure: a meta-analysis of double-blind, placebo-controlled, randomized trials. *Circulation* 98: 1184-1191, 1998.

32. **Leimbach WN, Jr., Wallin BG, Victor RG, Aylward PE, Sundlof G, and Mark AL**. Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. *Circulation* 73: 913-919, 1986.

33. **Magnusson G, Kaijser L, Sylven C, Karlberg KE, Isberg B, and Saltin B**. Peak skeletal muscle perfusion is maintained in patients with chronic heart failure when only a small muscle mass is exercised. *Cardiovasc Res* 33: 297-306, 1997.

34. **Mann DL**. *Heart failure : a companion to Braunwald's heart disease*. Philadelphia: Saunders, 2011, p. xix, 902 p.

35. **Massie B, Conway M, Yonge R, Frostick S, Ledingham J, Sleight P, Radda G, and Rajagopalan B**. Skeletal muscle metabolism in patients with congestive heart failure: relation to clinical severity and blood flow. *Circulation* 76: 1009-1019, 1987.

36. **Metra M, Raddino R, Dei Cas L, and Visioli O**. Assessment of peak oxygen consumption, lactate and ventilatory thresholds and correlation with resting and exercise hemodynamic data in chronic congestive heart failure. *Am J Cardiol* 65: 1127-1133, 1990.

37. **Middlekauff HR, Chiu J, Hamilton MA, Fonarow GC, Maclellan WR, Hage A, Moriguchi J, and Patel J**. Muscle mechanoreceptor sensitivity in heart failure. *Am J Physiol Heart Circ Physiol* 287: H1937-1943, 2004.

38. **Middlekauff HR, Nitzsche EU, Hoh CK, Hamilton MA, Fonarow GC, Hage A, and Moriguchi JD**. Exaggerated muscle mechanoreflex control of reflex renal vasoconstriction in heart failure. *J Appl Physiol (1985)* 90: 1714-1719, 2001.

39. **Middlekauff HR, Nitzsche EU, Nguyen AH, Hoh CK, and Gibbs GG**. Modulation of renal cortical blood flow during static exercise in humans. *Circ Res* 80: 62-68, 1997.

40. **Middlekauff HR, and Sinoway LI**. Increased mechanoreceptor stimulation explains the exaggerated exercise pressor reflex seen in heart failure. *J Appl Physiol (1985)* 102: 492-494; discussion 496, 2007.

41. **Ng AV, Callister R, Johnson DG, and Seals DR**. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. *Hypertension* 21: 498-503, 1993.

42. **Notarius CF, Ando S, Rongen GA, and Floras JS**. Resting muscle sympathetic nerve activity and peak oxygen uptake in heart failure and normal subjects. *Eur Heart J* 20: 880-887, 1999.

43. **Notarius CF, Atchison DJ, and Floras JS**. Impact of heart failure and exercise capacity on sympathetic response to handgrip exercise. *Am J Physiol Heart Circ Physiol* 280: H969-976, 2001.

44. **O'Leary DS, and Augustyniak RA**. Muscle metaboreflex increases ventricular performance in conscious dogs. *Am J Physiol* 275: H220-224, 1998.

45. **O'Leary DS, and Sheriff DD**. Is the muscle metaboreflex important in control of blood flow to ischemic active skeletal muscle in dogs? *Am J Physiol* 268: H980-986, 1995.

46. **Pawelczyk JA, Hanel B, Pawelczyk RA, Warberg J, and Secher NH**. Leg vasoconstriction during dynamic exercise with reduced cardiac output. *J Appl Physiol* 73: 1838-1846, 1992.

47. **Piepoli M, Clark AL, Volterrani M, Adamopoulos S, Sleight P, and Coats AJ**. Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. *Circulation* 93: 940-952, 1996.

48. **Piepoli M, Ponikowski P, Clark AL, Banasiak W, Capucci A, and Coats AJ**. A neural link to explain the "muscle hypothesis" of exercise intolerance in chronic heart failure. *Am Heart J* 137: 1050-1056, 1999.

49. **Piepoli MF, and Coats AJ**. Increased metaboreceptor stimulation explains the exaggerated exercise pressor reflex seen in heart failure. *J Appl Physiol (1985)* 102: 494-496; discussion 496-497, 2007.

50. **Remensnyder JP, Mitchell JH, and Sarnoff SJ**. Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res* 11: 370-380, 1962.

51. **Richardson RS, and Saltin B**. Human muscle blood flow and metabolism studied in the isolated quadriceps muscles. *Med Sci Sports Exerc* 30: 28-33, 1998.

52. **Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, and Turner MB**. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation* 125: e2-e220, 2012.

53. **Rosenmeier JB, Fritzlar SJ, Dinenno FA, and Joyner MJ**. Exogenous NO administration and alpha-adrenergic vasoconstriction in human limbs. *J Appl Physiol* 95: 2370-2374, 2003.

54. **Rosenmeier JB, Yegutkin GG, and Gonzalez-Alonso J**. Activation of ATP/UTP-selective receptors increases blood flow and blunts sympathetic vasoconstriction in human skeletal muscle. *J Physiol* 586: 4993-5002, 2008.

55. **Saltin B**. Hemodynamic adaptations to exercise. *Am J Cardiol* 55: 42D-47D, 1985.

56. **Secher NH, Clausen JP, Klausen K, Noer I, and Trap-Jensen J**. Central and regional circulatory effects of adding arm exercise to leg exercise. *Acta Physiol Scand* 100: 288-297, 1977.

57. **Shoemaker JK, Kunselman AR, Silber DH, and Sinoway LI**. Maintained exercise pressor response in heart failure. *J Appl Physiol (1985)* 85: 1793-1799, 1998.

58. **Shoemaker JK, Naylor HL, Hogeman CS, and Sinoway LI**. Blood flow dynamics in heart failure. *Circulation* 99: 3002-3008, 1999.

59. **Shoemaker JK, Pandey P, Herr MD, Silber DH, Yang QX, Smith MB, Gray K, and Sinoway LI**. Augmented sympathetic tone alters muscle metabolism with exercise: lack of evidence for functional sympatholysis. *J Appl Physiol* 82: 1932-1938, 1997.

60. **Silber DH, Sutliff G, Yang QX, Smith MB, Sinoway LI, and Leuenberger UA**. Altered mechanisms of sympathetic activation during rhythmic forearm exercise in heart failure. *J Appl Physiol* 84: 1551-1559, 1998.

61. **Smith EG, Voyles WF, Kirby BS, Markwald RR, and Dinenno FA**. Ageing and leg postjunctional alpha-adrenergic vasoconstrictor responsiveness in healthy men. *J Physiol* 582: 63-71, 2007.

62. **Sterns DA, Ettinger SM, Gray KS, Whisler SK, Mosher TJ, Smith MB, and Sinoway LI**. Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation* 84: 2034-2039, 1991.

63. **Sullivan MJ, and Hawthorne MH**. Exercise intolerance in patients with chronic heart failure. *Prog Cardiovasc Dis* 38: 1-22, 1995.

64. **Sundlof G, and Wallin BG**. Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J Physiol* 274: 621-637, 1978.

65. **Szlachcic J, Massie BM, Kramer BL, Topic N, and Tubau J**. Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. *Am J Cardiol* 55: 1037-1042, 1985.

66. **van der Harst P, and de Boer RA**. Statins in the treatment of heart failure. *Circ Heart Fail* 3: 462-464, 2010.

67. **Vasan RS, Larson MG, Benjamin EJ, Evans JC, Reiss CK, and Levy D**. Congestive heart failure in subjects with normal versus reduced left ventricular ejection fraction: prevalence and mortality in a population-based cohort. *J Am Coll Cardiol* 33: 1948-1955, 1999.

68. **Weber KT, Kinasewitz GT, Janicki JS, and Fishman AP**. Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. *Circulation* 65: 1213-1223, 1982.

69. **Wiener DH, Fink LI, Maris J, Jones RA, Chance B, and Wilson JR**. Abnormal skeletal muscle bioenergetics during exercise in patients with heart failure: role of reduced muscle blood flow. *Circulation* 73: 1127-1136, 1986.

70. **Wilkins BW, Schrage WG, Liu Z, Hancock KC, and Joyner MJ**. Systemic hypoxia and vasoconstrictor responsiveness in exercising human muscle. *J Appl Physiol* 101: 1343-1350, 2006.

71. **Wilson JR, and Ferraro N**. Effect of the renin-angiotensin system on limb circulation and metabolism during exercise in patients with heart failure. *J Am Coll Cardiol* 6: 556-563, 1985.

72. **Wilson JR, Ferraro N, and Wiener DH**. Effect of the sympathetic nervous system on limb circulation and metabolism during exercise in patients with heart failure. *Circulation* 72: 72-81, 1985.

73. **Wilson JR, Martin JL, and Ferraro N**. Impaired skeletal muscle nutritive flow during exercise in patients with congestive heart failure: role of cardiac pump dysfunction as determined by the effect of dobutamine. *Am J Cardiol* 53: 1308-1315, 1984.

74. **Wilson JR, Martin JL, Ferraro N, and Weber KT**. Effect of hydralazine on perfusion and metabolism in the leg during upright bicycle exercise in patients with heart failure. *Circulation* 68: 425-432, 1983.

75. **Wilson JR, Martin JL, Schwartz D, and Ferraro N**. Exercise intolerance in patients with chronic heart failure: role of impaired nutritive flow to skeletal muscle. *Circulation* 69: 1079-1087, 1984.

76. **Wilson JR, Wiener DH, Fink LI, and Ferraro N**. Vasodilatory behavior of skeletal muscle arterioles in patients with nonedematous chronic heart failure. *Circulation* 74: 775-779, 1986.

77. **Wray DW, Donato AJ, Nishiyama SK, and Richardson RS**. Acute sympathetic vasoconstriction at rest and during dynamic exercise in cyclists and sedentary humans. *J Appl Physiol (1985)* 102: 704-712, 2007.

78. **Wray DW, Fadel PJ, Smith ML, Raven P, and Sander M**. Inhibition of alpha-adrenergic vasoconstriction in exercising human thigh muscles. *J Physiol* 555: 545-563, 2004.

79. **Wray DW, Nishiyama SK, and Richardson RS**. Role of {alpha}1-adrenergic vasoconstriction in the regulation of skeletal muscle blood flow with advancing age. *Am J Physiol Heart Circ Physiol* 296: H497-504, 2009.

80. **Yin FCP, and Avolio AP**. *Ventricular/vascular coupling : clinical, physiological, and engineering aspects*. New York: Springer-Verlag, 1987, p. 406 p.

81. **Zelis R, Longhurst J, Capone RJ, and Mason DT**. A comparison of regional blood flow and oxygen utilization during dynamic forearm exercise in normal subjects and patients with congestive heart failure. *Circulation* 50: 137-143, 1974.

CHAPTER 2

METABORECEPTOR ACTIVATION IN HEART FAILURE WITH REDUCED EJECTION FRACTION: LINKING CENTRAL AND PERIPHERAL HEMODYNAMICS

Abstract

The present study sought to evaluate the metaboreflex in heart failure patients with reduced ejection fraction (HFrEF), with an emphasis on the interaction between central and peripheral hemodynamics. In 23 HFrEF patients (63 ± 2 yrs) and 15 healthy, controls of a similar age (64 ± 3 yrs), we examined mean arterial pressure (MAP), cardiac output (CO), systemic vascular conductance (SVC), effective arterial elastance (Ea), and stroke work (SW) during metaboreceptor activation elicited by postexercise circulatory occlusion following static-intermittent handgrip exercise (15, 30, and 45% of maximal voluntary contraction (MVC)). Across workloads, the metaboreflex-induced increase in MAP was similar between groups. In controls, this was driven by increases in CO (Δ495 ± 155, Δ564 ± 156, Δ666 ± 217 ml/min); however, in HFrEF, this change was accomplished by reductions in SVC (Δ–4.9 ± 1.5, Δ–9.1 ± 1.9, Δ–12.7 ± 1.8 ml/min/mmHg). This contributed to the exaggerated increases in Ea in HFrEF (Δ0.16 ± 0.05, Δ0.32 ± 0.07, Δ0.36 ± 0.07 mmHg/ml) compared to controls (Δ0.03 ± 0.05, Δ0.08 ± 0.07, Δ0.17 ± 0.06 mmHg/ml) which were associated with the attenuated increases in SW in this patient group (Δ3,247 ± 1,446 mmHg*ml / mmHg/ml) compared to controls (Δ13,853 ± 3,132 mmHg*ml / mmHg/ml). Together, these findings indicate a preserved role of the metaboreflex-induced pressor response in HFrEF, but suggest that this response is governed by the peripheral circulation in this cohort, a maladaptation that may exacerbate systolic dysfunction and contribute to exercise intolerance.

Introduction

Heart failure with reduced ejection fraction (HFrEF) is a clinical syndrome which is commonly linked to exercise intolerance and capacity (36, 40). While there are many contributing factors to the exercise limitations in this patient population, the role of skeletal muscle maladaptations has been increasingly recognized, with specific interest focused on the muscle metaboreflex. Activation of this reflex pathway is mediated by metabolically sensitive group IV afferent fibers (metaboreceptors) originating in skeletal muscle, which increase efferent sympathetic nervous system (SNS) activity in an effort to augment the perfusion of the exercising skeletal muscle through increases in arterial blood pressure (ABP) (2, 9, 25). Whether the metaboreflex is altered in HFrEF patients remains a topic of ongoing debate (22, 28), with evidence for both exaggerated (6, 20, 23, 34) and similar (26-28, 32, 33) reflex increases in mean arterial blood pressure (MAP) during metaboreflex activation. The disparate findings from these studies suggest that significant uncertainty remains regarding disease-related changes in the muscle metaboreflex in HFrEF patients, as well as the contribution of this reflex in the cardiovascular response to exercise.

Beyond the simple determination of the pressor response, further insight into the importance of metaboreceptor activation in patients with HFrEF may be gained by considering the relative contributions of changes in central (i.e. cardiac output (CO)) and peripheral (i.e. systemic vascular conductance (SVC)) hemodynamics to the increase in MAP in this patient group. Interestingly, in the animal model of heart failure (HF), the contributions of these factors to the overall metaboreflex-induced pressor response have been documented to be solely due to reductions in SVC across exercise intensities (13).

This is vastly different than the response observed in healthy animals, where the metaboreflex-induced increases in MAP were predominantly due to increases in CO at low to moderate intensities (25) and a shift towards a reliance on SVC to increase MAP only during high intensity exercise, when the ability to increase CO was compromised (4). In humans, only one study to date has examined central and peripheral contributions to the metaboreflex in HFrEF. Crisafulli *et al*. (9) reported a metaboreflex-induced increase in MAP which was predominantly driven by an increase in CO in healthy individuals and by a reduction in SVC in patients with HFrEF, suggesting a greater role of the peripheral vasculature in governing the pressor response. However, this study only included one level of metaboreceptor activation, leaving uncertainty regarding the graded nature of the response that has been demonstrated in an animal model of HF (13).

Whether the metaboreflex-mediated increase in MAP is achieved by central or peripheral mechanisms may be of particular significance in HFrEF patients due to the potential of this reflex to further stress cardiac muscle through substantial increases in afterload. Indeed, SVC, a measure of systemic vascular tone, represents the nonpulsatile component of arterial afterload (16, 41), and considering that patients with HFrEF are known to be afterload-sensitive (3, 15, 31), this group can experience severe impairments in left ventricular systolic function when arterial afterload is increased (15). Thus, while the metaboreflex response is typically viewed as an effective way to increase perfusion of the exercising muscle via increases in perfusion pressure in healthy individuals, this reflex may exacerbate existing ventricular dysfunction in HFrEF patients if a marked reduction in SVC is elicited upon metaboreceptor activation.

Thus, the purpose of this study was to implement the use of postexercise circulatory occlusion (PECO) following static-intermittent handgrip exercise across a range of exercise intensities to comprehensively investigate the interaction between central and peripheral responses to metaboreceptor activation. We hypothesized that: 1) HFrEF patients would exhibit similar increases in MAP across all levels of metaboreceptor activation compared to healthy controls, 2) HFrEF patients would exhibit a greater dependence on reductions in SVC than increases in CO to achieve the metaboreflex-induced pressor response, and 3) HFrEF patients would exhibit a greater increase in arterial afterload and an attenuated increase in functional systolic work in response to metaboreceptor activation compared to healthy controls.

Methods

Subjects

23 New York Heart Association (NYHA) class II-III HFrEF patients (22 males and 1 female) and 15 healthy, control subjects (14 males and 1 female) of a similar age were recruited either by word of mouth or in the HF clinics at the University of Utah Health Sciences Center and the Salt Lake City VA Medical Center. All control subjects were nonsmokers, not taking any prescription medication, and were free of overt cardiovascular disease, as indicated by a health history questionnaire. Protocol approval and written informed consent were obtained according to University of Utah and Salt Lake City Veterans Affairs Medical Center Institutional Review Board requirements. All data collection took place at the Utah Vascular Research Laboratory located at the Veterans Affairs Salt Lake City Geriatric, Research, Education, and Clinical Center. All

studies were performed in a thermoneutral environment, with subjects reporting to the laboratory fasted, and not having performed any exercise within 24 hours of the study.

Handgrip Exercise and Metaboreceptor Activation

Subjects were instrumented with a Finometer (Finapres Medical Systems, Amsterdam, the Netherlands) on the nonexercising arm, a 3-lead ECG (Biopac, Goleta, CA, U.S.A.) to measure heart rate, and a pneumatic blood pressure cuff distal to the antecubital fossa on the exercising arm to isolate the metaboreflex following exercise. Subjects then rested supine for ≈20 minutes. First, baseline measurements were taken over the course of 1-minute. Second, maximal voluntary contraction (MVC) was established by taking the highest value recorded of three maximal contractions using a handgrip dynamometer (TSD121C, Biopac Systems, Goleta, CA). Static-intermittent handgrip exercise was performed at three intensities based on each subject's respective MVC (15, 30, and 45% of MVC). The subjects squeezed the dynamometer to the sound of a metronome (60 beats/min) and real-time force output was displayed on a computer monitor so that subjects could monitor their effort and make corrections when necessary. Each bout of handgrip exercise lasted 3 minutes, and was followed by 2 minutes of forearm ischemia to isolate the metaboreflex, with measurements taken during the final minute. Forearm ischemia was achieved through the inflation of the pneumatic blood pressure cuff on the exercising arm to suprasystolic pressures (>250 mmHg) 5 seconds before the end of the handgrip exercise. A 5-minute recovery period was given after each period of metaboreceptor activation to allow cardiovascular variables to return to resting values. If cardiovascular variables did not return to resting values after 5 minutes, additional rest was given.

Measurements

Hemodynamic variables

Stroke volume (SV), heart rate (HR), CO, and ABP were determined noninvasively (Finapres Medical Systems BV, Amsterdam, The Netherlands). SV was calculated using the Modelflow method which includes age, sex, height, and weight in its algorithm (Beatscope version 1.1; Finapres Medical Systems BV, Amsterdam, The Netherlands) (5), and has been shown to accurately track SV during a variety of experimental protocols including exercise (10, 11, 35, 38). Pulse pressure (PP), a measure of pulsatile load, the nonresistive oscillatory component of arterial afterload (7, 18), was calculated as:

$$PP\ (mmHg) = systolic\ arterial\ pressure\ (SAP) - diastolic\ arterial\ pressure\ (DAP)$$

Total arterial compliance (TAC), an index of pulsatile arterial afterload which takes into account the effect of SV (7, 29), was calculated as:

$$TAC\ (ml/mmHg) = SV/PP$$

MAP was calculated as:

$$MAP\ (mmHg) = DAP + (PP*0.33)$$

End systolic arterial pressure (P_{es}) was calculated as (18):

$$P_{es} = 0.9*SAP.$$

CO was calculated as*:*

$$CO\ (L/min) = SV*HR$$

SVC, a measure of systemic vascular tone and the nonpulsatile (mean resistive) component of arterial afterload (16), was calculated as:

$$SVC\ (ml/min/mmHg) = CO/MAP$$

Systemic vascular resistance (SVR) was calculated as:

$$SVR\ (mmHg/L/min) = MAP/CO$$

Effective arterial elastance (Ea), an index of total arterial afterload (both pulsatile and nonpulsatile arterial afterload) (18, 29), was calculated as:

$$Ea\ (mmHg/ml) = P_{es}/SV$$

Stroke work (SW), a measure of functional systolic work performed by the left ventricle (16, 37), was calculated as:

$$SW\ (mmHg*ml) = P_{es}*SV$$

Rate pressure product (RPP), an index of myocardial oxygen consumption (19), was calculated as:

$$RPP\ (AU) = SAP*HR$$

Near infrared spectroscopy

To determine muscle microvascular deoxyhemoglobin (12) of one specific muscle group during exercise and metaboreceptor activation, in a subset of subjects (HFrEF = 13; control = 9), near infrared spectroscopy (NIRS) was employed on the belly of the brachioradialis and the flexor carpi radialis. A frequency-domain multidistance NIRS system was utilized (Oxiplex TS, ISS, Champaign, IL) that allows the absolute quantification of deoxyhemoglobin concentrations, expressed in μM (14). Prior to use, the probe was calibrated using a block with known absorption characteristics to calculate the absorption and scattering coefficients. Prior to placement, the skin covering the brachioradialis and the flexor carpi radialis was cleaned and double-sided adhesive tape was used to seat the diode, which was covered and further secured with coban (3M, St. Paul, MN). The data were acquired at 0.5 Hz, and 1-minute averages were calculated during the last minute of each exercise bout and during the final minute of PECO.

Data Analysis

Statistics were performed using commercially available software (SigmaStat 3.10; Systat Software, Point Richmond, CA). For both the exercise and metaboreceptor activation portion of the protocol, 2x4 repeated measures ANOVA ($\alpha < 0.05$) (group: 2 levels; controls vs. HFrEF) (workload or metaboreflex activation: 4 levels; rest, 15, 30, and 45% of MVC) were utilized to determine the exercise and metaboreflex-induced alterations in hemodynamic measurements. The Holm-Sidak method was used for alpha adjustment and post hoc analysis. Linear regression analysis was performed on individual data, with the slope and y-intercept determined to evaluate the associations between the metaboreflex-induced changes in SW with Ea and RPP. Student *t*-tests were used to

compare the effect of metaboreflex-induced changes on slope and y-intercept values. All group data are expressed as means ± SEM.

Results

Subject Characteristics

Baseline characteristics of the control subjects and HFrEF patients are displayed in **Table 2.1**. Disease-specific characteristics and medications of patients with HFrEF are presented in **Table 2.2**.

Rest and Exercise Hemodynamics

At rest, there were no significant differences in deoxyhemoglobin, MAP, CO, or SVC in HFrEF patients compared to controls **(Table 2.3)**. Exercise elicited similar intensity-dependent increases in deoxyhemoglobin and MAP between groups **(Table 2.3).** The changes in MAP were solely driven by increases in CO across workloads in control subjects **(Table 2.3)** and reductions in SVC in HFrEF patients **(Table 2.3)**. This was complemented by substantially attenuated increases in SAP and exaggerated increases in DAP in HFrEF compared to control subjects **(Table 2.3)**. These differences resulted in a lower PP across exercise intensities in HFrEF, compared to controls **(Table 2.3).** However, when factoring in the differences in SV on PP as expressed by TAC, there were significant intensity-dependent reductions in TAC observed in control subjects and only a significant reduction at the high workload in HFrEF. Additionally, there were no significant differences in TAC between groups at any workload **(Table 2.3**). Ea was significantly increased across all workloads in both groups; however, the increases were significantly greater in HFrEF patients compared to controls at the two highest workloads

(Table 2.3). SW increased significantly across all workloads in the control subjects, with no significant difference from rest demonstrated by the HFrEF patients **(Table 2.3)**. This was in light of similar RPP's across all workloads in both groups **(Table 2.3)**.

Metaboreflex-Induced Changes in Hemodynamics

Metaboreceptor activation provoked similar increases in tissue deoxyhemoglobin **(Table 2.4)** and MAP **(Figure 2.1, top and Table 2.4)** across increasing levels of metaboreceptor activation between groups. However, the metaboreflex-induced increases in MAP were due exclusively to increases in CO in the control group **(Figure 2.1, middle and Table 2.4)**, and reductions in SVC in HFrEF **(Figure 2.1, bottom and Table 2.4).** Similar to exercise, HFrEF patients exhibited a blunted increase in SAP across increasing levels of metaboreceptor activation and exaggerated increases in DAP compared to controls **(Table 2.4).** This led to a significantly attenuated increase PP in the HFrEF patients, who only established an increase in PP at the highest level of metaboreceptor activation **(Figure 2.2, top and Table 2.4)**. However, when factoring in the significantly greater increases in SV induced by the metaboreflex exhibited by the control subjects compared to HFrEF patients (expressed as TAC), TAC was not significantly different between groups at any level of metaboreceptor activation **(Figure 2.2, bottom and Table 2.4)**. Metaboreceptor activation provoked minimal increases in Ea in the control group, who only exhibited a significant increase at the highest level **(Figure 2.3 and Table 2.4)**. In contrast, HFrEF patients displayed a significant increase in Ea across all levels of metaboreceptor activation and were significantly different from control subjects at the highest two levels **(Figure 2.3 and Table 2.4)**. SW was significantly increased by metaboreceptor activation at every level of activation in the

control group, and only at the highest level in HFrEF patients **(Figure 2.4, top and Table 2.4)**. This contributed to the significantly lower SW across all levels of metaborceptor activation in HFrEF compared to controls **(Figure 2.4, top and Table 2.4).** These differences in SW between groups were due to significantly blunted changes in P_{es} **(Figure 2.4, middle)** and SV **(Figure 2.4, bottom and Table 2.4)**, induced by metaboreceptor activation in HFrEF patients compared to the control group. Across all levels of metaboreceptor activation, similar increases in RPP were observed between both groups **(Table 2.4).** The slope and the y-intercept of the relationships between metaboreflex-induced changes in SW and Ea were significantly greater in the control group compared to HFrEF **(Figure 2.5)**. This demonstrates that for a given metaboreflex-induced change in Ea, there was less of a metaboreflex-induced increase in SW in HFrEF compared to the control group. The slope of the relationship between the metaboreflex-induced changes in SW and RPP were not significantly different between groups **(Figure 2.6)**. However, the y-intercept was less in the HFrEF group compared to controls **(Figure 2.6)**, representing a downward shift in the relationship. This indicates that for a given metboreflex-induced change in RPP, there was less of a metaboreflex-induced increase in SW in HFrEF compared to the control group.

Discussion

The present study sought to comprehensively evaluate the muscle metaboreflex in HFrEF patients and healthy control subjects of a similar age, with an emphasis on investigating the central and peripheral hemodynamic contributions to the metaboreflex-induced pressor response. Across multiple levels of metaboreceptor activation, the increase in MAP was similar between groups, providing new evidence against a disease-

related exaggeration of the muscle metaboreflex-induced pressor response in HFrEF. However, a discrete pattern of central and peripheral hemodynamic changes was observed between groups. In control subjects, the pressor response induced by metaboreceptor activation was driven by increases in CO, with no significant changes in SVC. In contrast, progressively greater reductions in SVC contributed to the pressor response in HFrEF patients, while CO remained unchanged. This metaboreflex-induced reduction in SVC in HFrEF patients contributed to the reduction in Ea, which was associated with a blunted increase in the metaboreflex-induced changes in SW. Additionally, a downward shift in the relationship between the metaboreflex-induced changes in SW and RPP was observed in HFrE patients compared to control subjects, indicating a likely reduction in myocardial efficiency during metaboreceptor activation. Together, these findings indicate a preserved role of the muscle metaboreflex-induced pressor response in HFrEF. However, the shift to increases in peripheral vasoconstriction driving this response in HFrEF patients represents a maladaptive process which places a substantial hemodynamic load on the heart, potentially exacerbating the underlying impairment in systolic function and myocardial efficiency, and thereby contributing to the exercise limitations present in this patient group.

Metaboreflex Contribution to the Exercise-Induced Changes in Mean Arterial Pressure

It is well-established that patients with HFrEF suffer from a nearly insurmountable intolerance to physical exertion (36, 40), which may be due, at least in part, to maladaptations in skeletal muscle. Indeed, Drs. Coats and Piepoli have hypothesized that abnormalities in sensory reflex activity in skeletal muscle may

contribute to the exercise limitations in HFrEF (8, 27), the so-called "muscle hypothesis" of HF. Located within the skeletal muscle are two distinct sensory afferent fiber types; group III afferent fibers which are predominately mechanically sensitive (mechanoreceptors) and group IV afferent fibers (metaboreceptors) which are principally sensitive to metabolites produced during exercise (17). Collectively, these reflex pathways serve to increase SNS activity, which ultimately increase perfusion pressure (2, 9, 25). In HF patients, some aspect of this reflex response appears to be dysfunctional.

While difficult to completely isolate these respective reflex pathways, PECO has become a widely adopted approach whereby metabolic byproducts produced during exercise are trapped distal to the point of occlusion, thereby activating group IV afferent fibers with minimal input from group III fibers (1). Despite extensive use of this experimental technique over the past 80 years in both healthy humans and patient populations, the exact role of metaboreceptor activation in the cardiovascular response to exercise in HFrEF remains a topic of ongoing debate. Indeed, studies which have investigated the pressor response during PECO have documented both exaggerated (27, 32) and similar (6, 20, 23, 34) increases in MAP in HFrEF patients compared to healthy individuals. The conflicting findings from these studies indicate that significant uncertainty remains as to whether the metaboreflex-induced pressor response is altered in HFrEF.

In the present study, we employed the PECO technique following three different handgrip exercise intensities in an effort to comprehensively evaluate the muscle metaboreflex in HFrEF patients compared to healthy control subjects of a similar age. As displayed in **Figure 2.1 (top)**, we observed a metaboreflex-induced pressor response that

was almost identical between groups across all levels of metaboreceptor activation. These results are in disagreement with some of the earliest work on the topic (27, 32), and may be explained by differences in experimental protocols, including differing handgrip exercise paradigms and methods of activating the muscle metaboreflex (PECO vs. limb positive pressure). To our knowledge, this is the first study to perform PECO following multiple intensities of static-intermittent handgrip exercise, providing a systematic assessment of the pressor response across multiple levels of metaboreceptor activation. The present findings thus extend on the observations from previous work (6, 20, 23, 34), providing new evidence against a disease-related exaggeration of the pressor response induced by the muscle metaboreflex in HFrEF patients.

Central and Peripheral Hemodynamic Contributions to Metaboreflex-Induced Changes in Mean Arterial Pressure

While a large breadth of research has focused on elucidating the strength of the metaboreflex-induced pressor response in HFrEF, limited work has been undertaken to examine variables contributing to this rise in MAP. In a healthy animal model, O'Leary *et al.* (25) documented that the rise in MAP triggered by metaboreceptor activation was solely due to increases in CO during low to moderate exercise intensities. Subsequent work from the same group reported that the metaboreflex-induced increases in MAP in an animal model of HF were primarily due to reductions in SVC (13), indicating that the pressor response was achieved exclusively via peripheral vasoconstriction. Based on these findings, the authors concluded that the inability of the metaboreflex to increase CO

in HF is detrimental as a reduction in SVC is the sole variable this reflex pathway can alter in order to increase ABP and ultimately blood flow to exercising skeletal muscle.

The present study extends these previous findings in animals to human HF, documenting metaboreflex-induced increases in MAP in patients with HFrEF **(Figure 2.1, top)** that were primarily accomplished through reductions in SVC **(Figure 2.1, bottom**), with virtually no changes in CO **(Figure 2.1, middle**). This was in marked contrast to the response observed in healthy control subjects, where increases in CO played a dominant role in increasing MAP during metaboreceptor activation **(Figure 2.1, middle)**. To our knowledge, only one other study in humans has examined the roles of CO and SVC in increasing MAP during metaboreceptor activation in patients with HFrEF. Crisafulli *et al*. (9) reported a metaboreflex-induced increase in MAP which was predominantly driven by an increase in CO in healthy individuals and by a reduction in SVC in patients with HFrEF, suggesting a greater role of the peripheral vasculature in governing the metaboreflex-induced pressor response in the patient group. However, this previous study only investigated the hemodynamic alterations induced by one level of metaboreceptor activation, which somewhat limits interpretation. The importance of examining multiple levels of metaboreceptor activation should not be underestimated; in the animal model, both CO and SVC responses to metaboreceptor activation differed significantly with increasing exercise intensity (4, 25). The present study thus provides the first comprehensive investigation into the role of CO and SVC in the metaboreflex-induced pressor response in HFrEF in humans, identifying an intensity-dependent reduction in SVC during metaboreflex activation in HFrEF, and thus indicating a proportionally greater role of SVC in increasing MAP in this patient group.

Arterial Afterload and Systolic Function

The manner by which metaboreceptor activation elicits an increase in MAP may be particularly significant when considering the relationship between the heart and the peripheral vasculature in HFrEF patients. Indeed, SVC represents the nonpulsatile component of arterial afterload (16, 41) and it is well-established that patients with HFrEF are afterload-sensitive (3, 15, 31) and face certain impairment in left ventricular systolic function if arterial afterload is increased (15). In the present study, at all levels of metaboreceptor activation, HFrEF patients exhibited an exaggerated increase in Ea, an index of total arterial afterload, compared to control subjects who only exhibited a significant augmentation in Ea at the highest level of metaboreceptor activation **(Figure 2.3)**. Due to the lack of differences between groups in the reduction in TAC, a measure of the pulsatile component of arterial afterload **(Figure 2.2, bottom)**, SVC likely is the primary contributor to the exaggerated increase in Ea and total arterial afterload induced by metaboreceptor activation exhibited in HFrEF. To our knowledge, this is the first study to definitively document an augmented arterial afterload induced by the metaboreflex-driven changes in SVC in HFrEF.

This metaboreflex-induced increase in arterial afterload in HFrEF appears to have deleterious cardiac effects. In HFrEF patients, metaboreceptor activation provoked much smaller increases in SW (a measure of functional systolic work) compared to healthy control subjects (**Figure 2.4, top**), and it appears that arterial afterload may have contributed significantly to this response. Indeed, the attenuated slope and the downward shift in the relationship between metaboreflex-induced changes in SW, with the reflex changes in Ea **(Figure 2.5)**, indicates a marked role of arterial afterload as a primary

restraining factor to the ability of the metaboreflex to increase CO and ultimately perfusion pressure in HFrEF. Compounded with these impairments in systolic function, HFrEF patients exhibited a reduction in myocardial efficiency, as estimated by the downward shift in the relationship between SW and RPP in HFrEF compared to control subjects (**Figure 2.6**). Taken together, these cardiac indices suggest that the metaboreflex-induced reductions in SVC and associated increases in afterload come at a steep cost to HFrEF patients, comprised of both reductions in systolic work and myocardial efficiency.

Experimental Considerations

The PECO technique is based on the principle that metabolites produced by the exercising skeletal muscle become trapped during circulatory occlusion, maintaining activation of the muscle metaboreflex. Though this well-established experimental approach provides a reproducible, intensity-dependent pressor response (**Figure 2.1**), we recognize the possibility that the metabolic milieu in the occluded tissue may differ between groups. However, this concern is somewhat mitigated by the inclusion of NIRS measurements of the occluded muscle, which indicated a similar level of muscle ischemia (as quantified by changes in deoxyhemoglobin) between HFrEF and control groups during all levels of metaboreceptor activation (**Table 2.4**). Additionally, we acknowledge that the arterial afterload calculations used in the current study are typically based on ABP measurements taken centrally, at the aorta (18). Due to the invasive nature and feasibility of collecting central aortic pressures in a large cohort of subjects, in the current study, ABP measurements were obtained noninvasively via finger photoplethysmography. Peripheral ABP measurements are often thought to not

completely describe central ABP due to the documented ABP wave amplification descending the arterial tree (30, 39). However, central ABP may also be augmented due to reflected pressure waveforms (30), therefore limiting the discrepancy in central and peripheral ABP. Indeed, prior studies suggest that P_{es}, when calculated using ABP measured peripherally, closely approximates central P_{es}, overestimating central P_{es} by less than 5% (18, 21, 24).

Conclusions

We have identified a preserved role of the metaboreflex-induced pressor response in HFrEF patients, and provide evidence that the rise in MAP is governed almost entirely by the peripheral circulation in this cohort. The net effect of this response appears to be maladaptive, as it places a substantial hemodynamic load on the heart, exacerbates the underlying impairment of systolic function, and likely contributes to exercise intolerance in this patient group.

References

1. **Alam M, and Smirk FH**. Observations in man upon a blood pressure raising reflex arising from the voluntary muscles. *J Physiol* 89: 372-383, 1937.

2. **Amann M, Runnels S, Morgan DE, Trinity JD, Fjeldstad AS, Wray DW, Reese VR, and Richardson RS**. On the contribution of group III and IV muscle afferents to the circulatory response to rhythmic exercise in humans. *J Physiol* 589: 3855-3866, 2011.

3. **Asanoi H, Sasayama S, and Kameyama T**. Ventriculoarterial coupling in normal and failing heart in humans. *Circ Res* 65: 483-493, 1989.

4. **Augustyniak RA, Collins HL, Ansorge EJ, Rossi NF, and O'Leary DS**. Severe exercise alters the strength and mechanisms of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol* 280: H1645-1652, 2001.

5. **Bogert LW, and van Lieshout JJ**. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Exp Physiol* 90: 437-446, 2005.

6. **Carrington CA, Fisher WJ, Davies MK, and White MJ**. Muscle afferent and central command contributions to the cardiovascular response to isometric exercise of postural muscle in patients with mild chronic heart failure. *Clin Sci (Lond)* 100: 643-651, 2001.

7. **Chemla D, Hebert JL, Coirault C, Zamani K, Suard I, Colin P, and Lecarpentier Y**. Total arterial compliance estimated by stroke volume-to-aortic pulse pressure ratio in humans. *Am J Physiol* 274: H500-505, 1998.

8. **Coats AJ, Clark AL, Piepoli M, Volterrani M, and Poole-Wilson PA**. Symptoms and quality of life in heart failure: the muscle hypothesis. *Br Heart J* 72: S36-39, 1994.

9. **Crisafulli A, Salis E, Tocco F, Melis F, Milia R, Pittau G, Caria MA, Solinas R, Meloni L, Pagliaro P, and Concu A**. Impaired central hemodynamic response and exaggerated vasoconstriction during muscle metaboreflex activation in heart failure patients. *Am J Physiol Heart Circ Physiol* 292: H2988-2996, 2007.

10. **de Vaal JB, de Wilde RB, van den Berg PC, Schreuder JJ, and Jansen JR**. Less invasive determination of cardiac output from the arterial pressure by aortic diameter-calibrated pulse contour. *Br J Anaesth* 95: 326-331, 2005.

11. **de Wilde RB, Geerts BF, Cui J, van den Berg PC, and Jansen JR**. Performance of three minimally invasive cardiac output monitoring systems. *Anaesthesia* 64: 762-769, 2009.

12. **DeLorey DS, Kowalchuk JM, and Paterson DH**. Relationship between pulmonary O2 uptake kinetics and muscle deoxygenation during moderate-intensity exercise. *J Appl Physiol (1985)* 95: 113-120, 2003.

13. **Hammond RL, Augustyniak RA, Rossi NF, Churchill PC, Lapanowski K, and O'Leary DS**. Heart failure alters the strength and mechanisms of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol* 278: H818-828, 2000.

14. **Hueber DM, Franceschini MA, Ma HY, Zhang Q, Ballesteros JR, Fantini S, Wallace D, Ntziachristos V, and Chance B**. Non-invasive and quantitative near-infrared haemoglobin spectrometry in the piglet brain during hypoxic stress, using a frequency-domain multidistance instrument. *Phys Med Biol* 46: 41-62, 2001.

15. **Kameyama T, Asanoi H, Ishizaka S, and Sasayama S**. Ventricular load optimization by unloading therapy in patients with heart failure. *J Am Coll Cardiol* 17: 199-207, 1991.

16. **Kass DA, and Kelly RP**. Ventriculo-arterial coupling: concepts, assumptions, and applications. *Ann Biomed Eng* 20: 41-62, 1992.

17. **Kaufman MP, and Hayes SG**. The exercise pressor reflex. *Clin Auton Res* 12: 429-439, 2002.

18. **Kelly RP, Ting CT, Yang TM, Liu CP, Maughan WL, Chang MS, and Kass DA**. Effective arterial elastance as index of arterial vascular load in humans. *Circulation* 86: 513-521, 1992.

19. **Kitamura K, Jorgensen CR, Gobel FL, Taylor HL, and Wang Y**. Hemodynamic correlates of myocardial oxygen consumption during upright exercise. *J Appl Physiol* 32: 516-522, 1972.

20. **Kon H, Nakamura M, Arakawa N, and Hiramori K**. Muscle metaboreflex is blunted with reduced vascular resistance response of nonexercised limb in patients with chronic heart failure. *J Card Fail* 10: 503-510, 2004.

21. **Kroeker EJ, and Wood EH**. Comparison of simultaneously recorded central and peripheral arterial pressure pulses during rest, exercise and tilted position in man. *Circ Res* 3: 623-632, 1955.

22. **Middlekauff HR, and Sinoway LI**. Increased mechanoreceptor stimulation explains the exaggerated exercise pressor reflex seen in heart failure. *J Appl Physiol (1985)* 102: 492-494; discussion 496, 2007.

23. **Notarius CF, Atchison DJ, and Floras JS**. Impact of heart failure and exercise capacity on sympathetic response to handgrip exercise. *Am J Physiol Heart Circ Physiol* 280: H969-976, 2001.

24. **Nussbacher A, Gerstenblith G, O'Connor FC, Becker LC, Kass DA, Schulman SP, Fleg JL, and Lakatta EG**. Hemodynamic effects of unloading the old heart. *Am J Physiol* 277: H1863-1871, 1999.

25. **O'Leary DS, and Augustyniak RA**. Muscle metaboreflex increases ventricular performance in conscious dogs. *Am J Physiol* 275: H220-224, 1998.

26. **Piepoli M, Clark AL, Volterrani M, Adamopoulos S, Sleight P, and Coats AJ**. Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. *Circulation* 93: 940-952, 1996.

27. **Piepoli M, Ponikowski P, Clark AL, Banasiak W, Capucci A, and Coats AJ**. A neural link to explain the "muscle hypothesis" of exercise intolerance in chronic heart failure. *Am Heart J* 137: 1050-1056, 1999.

28. **Piepoli MF, and Coats AJ**. Increased metaboreceptor stimulation explains the exaggerated exercise pressor reflex seen in heart failure. *J Appl Physiol (1985)* 102: 494-496; discussion 496-497, 2007.

29. **Reil JC, Tardif JC, Ford I, Lloyd SM, O'Meara E, Komajda M, Borer JS, Tavazzi L, Swedberg K, and Bohm M**. Selective heart rate reduction with ivabradine unloads the left ventricle in heart failure patients. *J Am Coll Cardiol* 62: 1977-1985, 2013.

30. **Safar ME, Protogerou AD, and Blacher J**. Statins, central blood pressure, and blood pressure amplification. *Circulation* 119: 9-12, 2009.

31. **Schwartzenberg S, Redfield MM, From AM, Sorajja P, Nishimura RA, and Borlaug BA**. Effects of vasodilation in heart failure with preserved or reduced ejection fraction implications of distinct pathophysiologies on response to therapy. *J Am Coll Cardiol* 59: 442-451, 2012.

32. **Shoemaker JK, Kunselman AR, Silber DH, and Sinoway LI**. Maintained exercise pressor response in heart failure. *J Appl Physiol (1985)* 85: 1793-1799, 1998.

33. **Silber DH, Sutliff G, Yang QX, Smith MB, Sinoway LI, and Leuenberger UA**. Altered mechanisms of sympathetic activation during rhythmic forearm exercise in heart failure. *J Appl Physiol* 84: 1551-1559, 1998.

34. **Sterns DA, Ettinger SM, Gray KS, Whisler SK, Mosher TJ, Smith MB, and Sinoway LI**. Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation* 84: 2034-2039, 1991.

35. **Sugawara J, Tanabe T, Miyachi M, Yamamoto K, Takahashi K, Iemitsu M, Otsuki T, Homma S, Maeda S, Ajisaka R, and Matsuda M**. Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between

Modelflow method and Doppler echocardiography method. *Acta Physiol Scand* 179: 361-366, 2003.

36. **Sullivan MJ, and Hawthorne MH**. Exercise intolerance in patients with chronic heart failure. *Prog Cardiovasc Dis* 38: 1-22, 1995.

37. **Sunagawa K, Maughan WL, and Sagawa K**. Optimal arterial resistance for the maximal stroke work studied in isolated canine left ventricle. *Circ Res* 56: 586-595, 1985.

38. **van Lieshout JJ, Toska K, van Lieshout EJ, Eriksen M, Walloe L, and Wesseling KH**. Beat-to-beat noninvasive stroke volume from arterial pressure and Doppler ultrasound. *Eur J Appl Physiol* 90: 131-137, 2003.

39. **Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, Hughes AD, Thurston H, and O'Rourke M**. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 113: 1213-1225, 2006.

40. **Wilson JR, Martin JL, Ferraro N, and Weber KT**. Effect of hydralazine on perfusion and metabolism in the leg during upright bicycle exercise in patients with heart failure. *Circulation* 68: 425-432, 1983.

41. **Yin FCP, and Avolio AP**. *Ventricular/vascular coupling : clinical, physiological, and engineering aspects*. New York: Springer-Verlag, 1987, p. 406 p.

Figure 2.1 Metaboreflex-induced changes in mean arterial pressure (*top*), cardiac output (*middle*), and systemic vascular conductance (*bottom*) in control subjects and heart failure patients with reduced ejection fraction (HFrEF). ***** Significant difference from control, $P<0.05$; † Significant difference from rest, $P<0.05$.

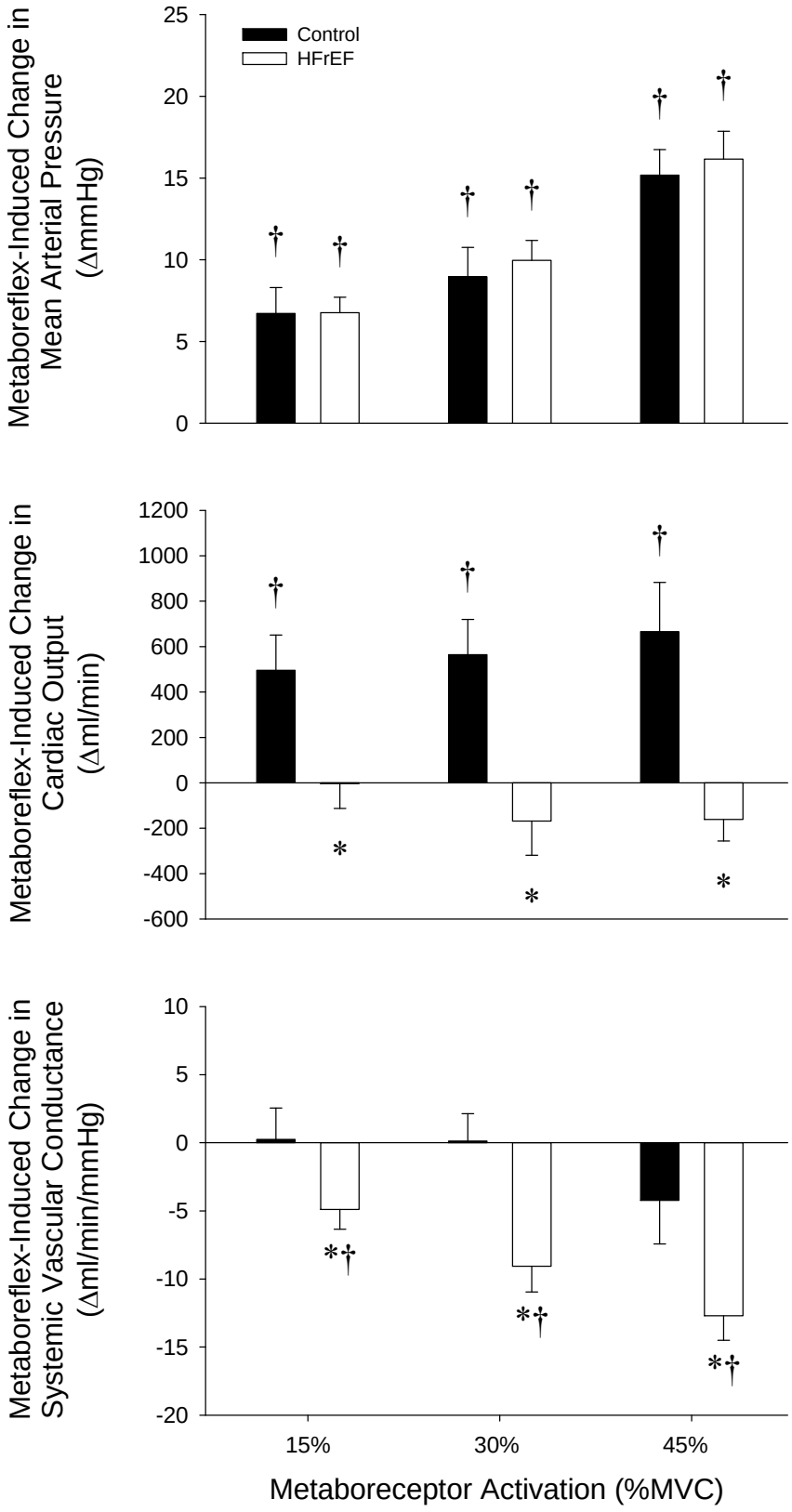

Metaboreflex-Induced Change in Mean Arterial Pressure (ΔmmHg)
Control
HFrEF
25
20
15
10
5
0
†
Metaboreflex-Induced Change in Cardiac Output (Δml/min)
1200
1000
800
600
400
200
0
-200
-400
-600
*
Metaboreflex-Induced Change in Systemic Vascular Conductance (Δml/min/mmHg)
10
5
0
-5
-10
-15
-20
*†
15%
30%
45%
Metaboreceptor Activation (%MVC)

Figure 2.2 Metaboreflex-induced changes in pulse pressure (*top*) and total arterial compliance (*bottom*) in control subjects and heart failure patients with reduced ejection fraction (HFrEF). * Significant difference from control, $P<0.05$; † Significant difference from rest, $P<0.05$.

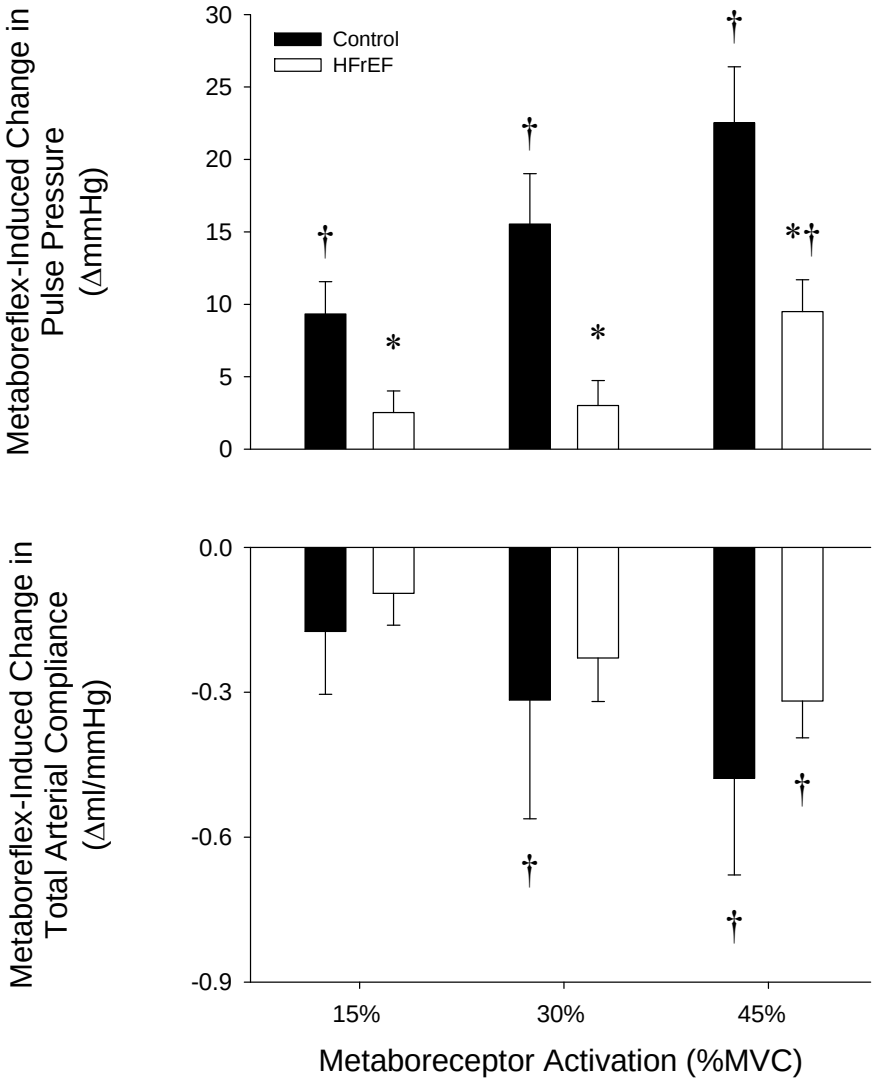

Metaboreflex-Induced Change in Pulse Pressure (ΔmmHg)
Control
HFrEF
30
25
20
15
10
5
0
†
*
†
*
†
*†
Metaboreflex-Induced Change in Total Arterial Compliance (Δml/mmHg)
0.0
-0.3
-0.6
-0.9
†
†
†
15%
30%
45%
Metaboreceptor Activation (%MVC)

Figure 2.3 Metaboreflex-induced changes in effective arterial elastance in control subjects and heart failure patients with reduced ejection fraction (HFrEF). * Significant difference from control, $P<0.05$; † Significant difference from rest, $P<0.05$.

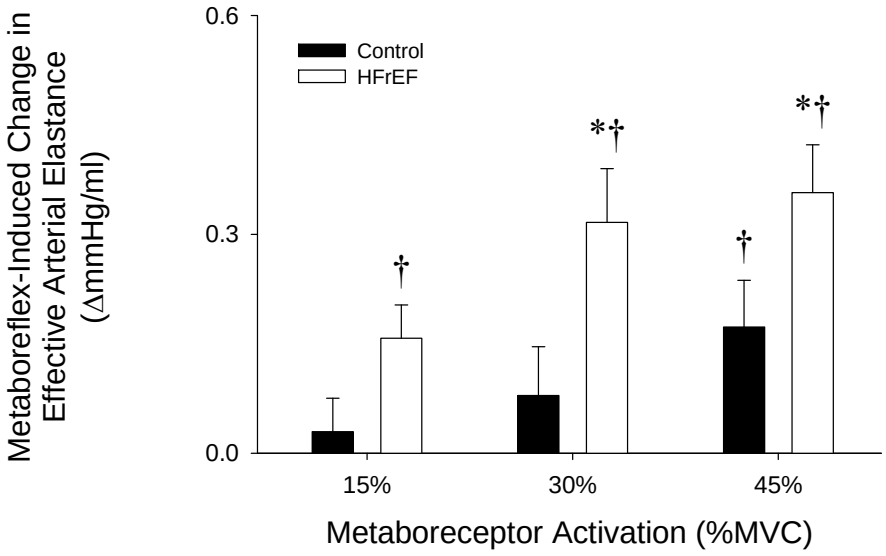

Metaboreflex-Induced Change in Effective Arterial Elastance (ΔmmHg/ml)
0.6
0.3
0.0
Control
HFrEF
*†
*†
†
†
15%
30%
45%
Metaboreceptor Activation (%MVC)

Figure 2.4 Metaboreflex-induced changes in stroke work (*top*), end systolic pressure (*middle*), and stroke volume (*bottom*) in control subjects and heart failure patients with reduced ejection fraction (HFrEF). * Significant difference from control, $P<0.05$; † Significant difference from rest, $P<0.05$.

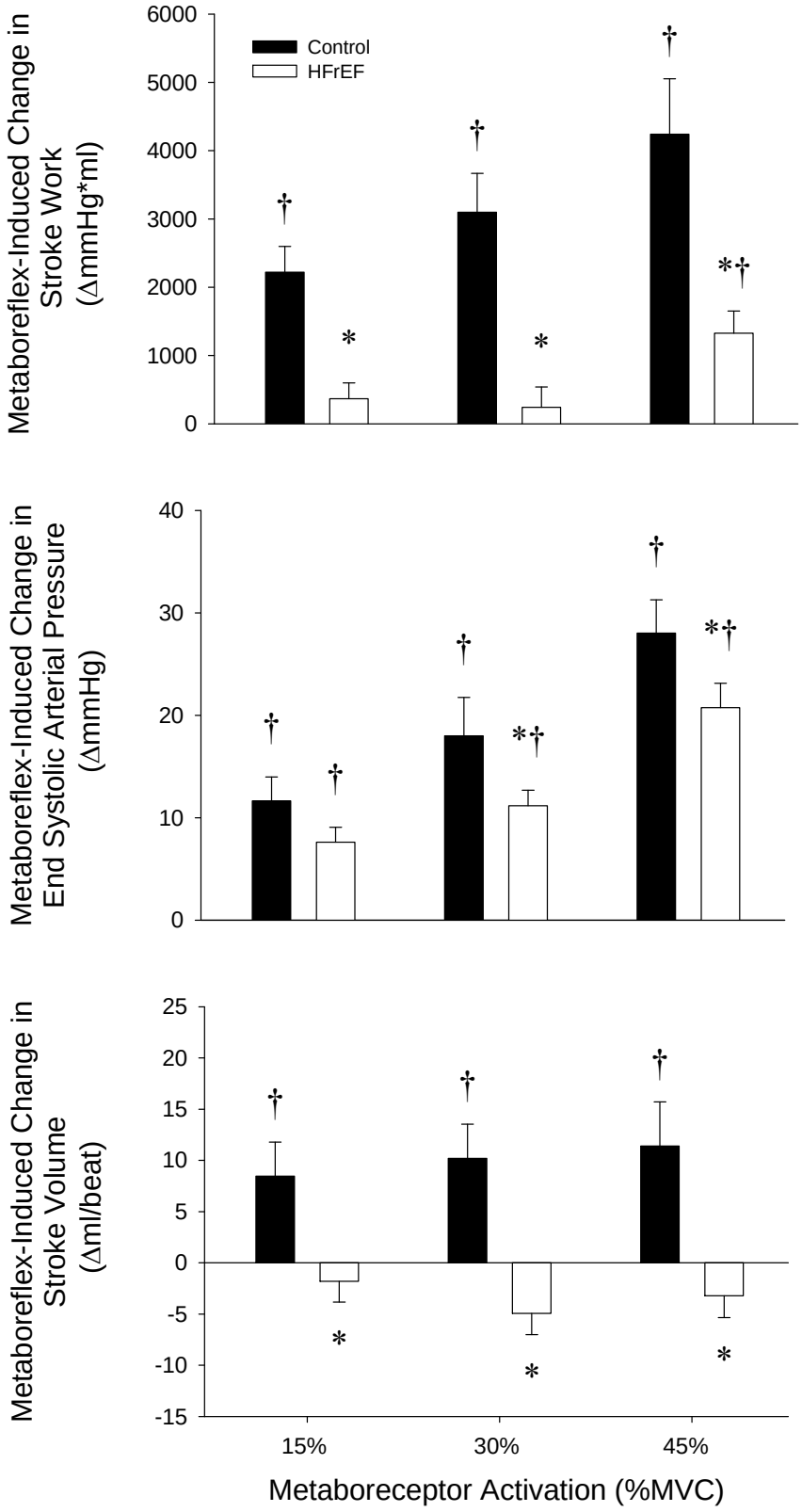

Metaboreflex-Induced Change in Stroke Work (ΔmmHg*ml)
6000
5000
4000
3000
2000
1000
0
Control
HFrEF
†
*
*†
Metaboreflex-Induced Change in End Systolic Arterial Pressure (ΔmmHg)
40
30
20
10
0
Metaboreflex-Induced Change in Stroke Volume (Δml/beat)
25
20
15
10
5
0
-5
-10
-15
15%
30%
45%
Metaboreceptor Activation (%MVC)

Figure 2.5 Relationship between metaboreflex-induced changes in stroke work and changes in effective arterial elastance in control subjects and heart failure patients with reduced ejection fraction (HFrEF). ***** Significant difference from control, $P<0.05$.

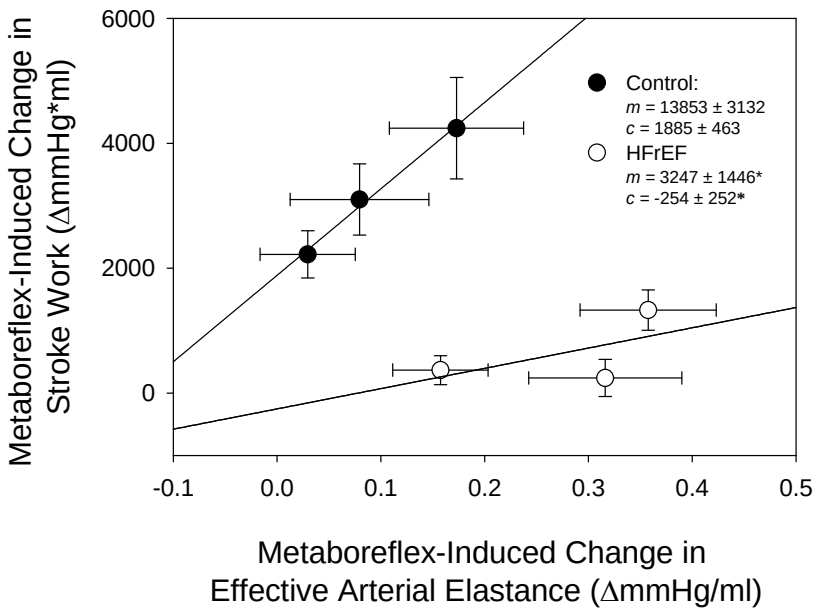

Metaboreflex-Induced Change in Stroke Work (ΔmmHg*ml)
6000
4000
2000
0
-0.1
0.0
0.1
0.2
0.3
0.4
0.5
Control:
m = 13853 ± 3132
c = 1885 ± 463
HFrEF
m = 3247 ± 1446*
c = -254 ± 252*
Metaboreflex-Induced Change in
Effective Arterial Elastance (ΔmmHg/ml)

Figure 2.6 Relationship between metaboreflex-induced changes in stroke work and changes in rate pressure product in control subjects and heart failure patients with reduced ejection fraction (HFrEF). ***** Significant difference from control, $P<0.05$.

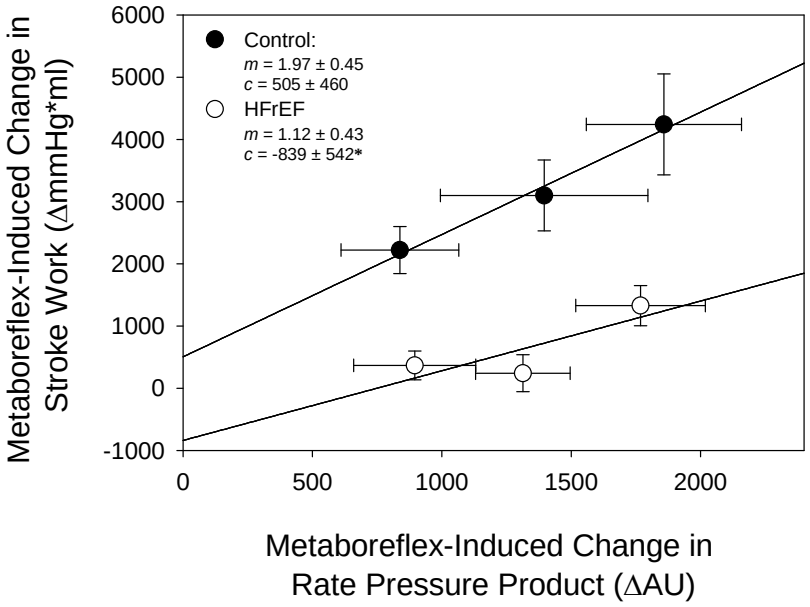

Metaboreflex-Induced Change in Stroke Work (ΔmmHg*ml)
6000
5000
4000
3000
2000
1000
0
-1000
0
500
1000
1500
2000
Control:
m = 1.97 ± 0.45
c = 505 ± 460
HFrEF
m = 1.12 ± 0.43
c = -839 ± 542*
Metaboreflex-Induced Change in Rate Pressure Product (ΔAU)

Table 2.1 *Subject characteristics*

	Control (n = 15)	HFrEF (n = 23)
Age, yrs	64 ± 3	63 ± 2
Height, cm	176 ± 2	175 ± 1
Weight, kg	80 ± 4	85 ± 4
Body mass index, kg/m^2	26 ± 1	28 ± 1
Maximum voluntary contraction, kg	27 ± 2	25 ± 2
Glucose, mg/dl	85 ± 6	99 ± 4
Total cholesterol, mg/dl	192 ± 14	155 ± 11
Triglycerides, mg/dl	143 ± 28	131 ± 12
HDL, mg/dl	49 ± 4	39 ± 2*
LDL, mg/dl	124 ± 11	96 ± 8

HFrEF, heart failure with reduced ejection fraction; HDL, high density lipoprotein; LDL, low density lipoprotein. Data are expressed as means ± SEM. * Significant difference from control, $P < 0.05$.

Table 2.2 *Disease - specific characteristics and medications*

	HFrEF (n = 23)
Disease-specific characteristics	
Left ventricular ejection fraction, % (means ± SEM)	22 ± 3
Diagnosis (ischemic)	14 / 23
Diagnosis (nonischemic)	9 / 23
NYHA class II	16 / 23
NYHA class III	7 / 23
Diabetic	4 / 23
Medications	
β-Blocker	23 / 23
ACE inhibitor	17 / 23
Angiotensin receptor inhibitor	4 / 23
Statin	18 / 23
Diuretic	18 / 23
Aldosterone inhibitor	4 / 23
Calcium channel inhibitor	1 / 23
Digoxin	4 / 23
Anticoagulant	13 / 23
Antiarrhythmic	1 / 23
Erythropoiesis - stimulating agent	1 / 23

HFrEF, heart failure with reduced ejection fraction; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme.

Table 2.3 *Central and peripheral hemodynamics at rest and during exercise*

Workload (%MVC)	Rest	15%	30%	45%
Control				
Mean arterial pressure, mmHg	83 ± 2	92 ± 2†	96 ± 3†	103 ± 2†
Systolic arterial pressure, mmHg	119 ± 3	140 ± 4†	143 ± 5†	154 ± 3
Diastolic arterial pressure, mmHg	66 ± 2	68 ± 2	73 ± 2†	77 ± 2†
Pulse pressure, mmHg	53 ± 3	71 ± 4†	70 ± 4†	77 ± 3†
Heart rate, beats/min	57 ± 2	64 ± 2†	65 ± 2†	68 ± 3†
Stroke volume, ml/beat	109 ± 5	110 ± 5	111 ± 4	110 ± 4
Cardiac output, L/min	6.3 ± 0.3	7.0 ± 0.4†	7.2 ± 0.4†	7.4 ± 0.4†
Systemic vascular conductance, ml/min/mmHg	75 ± 4	77 ± 5	75 ± 4	72 ± 4
Systemic vascular resistance, mmHg/L/min	14 ± 1	14 ± 1	14 ± 1	14 ± 1
Total arterial compliance, ml/mmHg	2.2 ± 0.2	1.6 ± 0.1†	1.7 ± 0.2†	1.5 ± 0.1†
Effective arterial elastance, mmHg/ml	1.0 ± 0.0	1.2 ± 0.0†	1.2 ± 0.1†	1.3 ± 0.1†
Stroke work, mmHg*ml	11,700 ± 592	13,920 ± 813†	14,357 ± 837†	15,308 ± 683†
Rate pressure product, AU	6,821 ± 254	8,924 ± 339†	9,354 ± 513†	10,417 ± 440†
Deoxyhemoglobin, μM (n=9)	27 ± 3	34 ± 4†	37 ± 6†	39 ± 5†
HFrEF				
Mean arterial pressure, mmHg	84 ± 3	93 ± 3†	95 ± 4†	102 ± 3†
Systolic arterial pressure, mmHg	117 ± 4	128 ± 5†	131 ± 5†	141 ± 5*†
Diastolic arterial pressure, mmHg	67 ± 3	75 ± 3†	77 ± 3 †	83 ± 3†
Pulse pressure, mmHg	50 ± 3	53 ± 3*	53 ± 4*	58 ± 4*†
Heart rate, beats/min	67 ± 2*	69 ± 3†	71 ± 2†	73 ± 3†
Stroke volume, ml/beat	83 ± 4*	81 ± 4*	79 ± 4*	76 ± 4*
Cardiac output, L/min	5.4 ± 0.3	5.5 ± 0.2*	5.6 ± 0.3*	5.4 ± 0.3*
Systemic vascular conductance, ml/min/mmHg	68 ± 4	62 ± 3*†	61 ± 4*†	55 ± 3*†
Systemic vascular resistance, mmHg/L/min	17 ± 2	18 ± 2†	20 ± 3*†	21 ± 2*†
Total arterial compliance, ml/mmHg	1.8 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.1†
Effective arterial elastance, mmHg/ml	1.4 ± 0.1	1.6 ± 0.1†	1.6 ± 0.1*†	1.8 ± 0.2*†
Stroke work, mmHg*ml	8,654 ± 464	9,253 ± 469*	9,192 ± 529*	9,562 ± 646*
Rate pressure product, AU	7,677 ± 310	8,902 ± 450†	9,215 ± 455†	10,322 ± 578†
Deoxyhemoglobin, μM (n=13)	28 ± 2	32 ± 2†	35 ± 2†	36 ± 3†

MVC, maximum voluntary contraction; HFrEF, heart failure with reduced ejection fraction. Data are expressed as means ± SEM.
* Significant difference from control, P<0.05; † Significant difference from rest, P<0.05.

Table 2.4 *Central and peripheral hemodynamics at rest and during metaboreceptor activation*

Metaboreceptor activation	Rest	15%	30%	45%
Control				
Mean arterial pressure, mmHg	83 ± 2	90 ± 3†	93 ± 3†	100 ± 2†
Systolic arterial pressure, mmHg	119 ± 3	132 ± 5†	139 ± 5†	150 ± 5†
Systolic arterial pressure, ΔmmHg	-	13 ± 3†	19 ± 3†	34 ± 4†
Diastolic arterial pressure, mmHg	66 ± 2	69 ± 2†	70 ± 2†	74 ± 1†
Diastolic arterial pressure, ΔmmHg	-	4 ± 1†	4 ± 1†	8 ± 1†
Pulse pressure, mmHg	53 ± 3	62 ± 3†	68 ± 4†	75 ± 5†
Heart rate, beats/min	57 ± 2	58 ± 2	59 ± 2	58 ± 2
Stroke volume, ml/beat	109 ± 5	118 ± 5†	118 ± 5†	118 ± 5†
Cardiac output, L/min	6.3 ± 0.3	6.8 ± 0.3†	6.9 ± 0.3†	6.8 ± 0.3†
Systemic vascular conductance, ml/min/mmHg	75 ± 4	76 ± 3	75 ± 3	68 ± 3†
Systemic vascular resistance, mmHg/L/min	14 ± 1	14 ± 1	14 ± 1	15 ± 1
Total arterial compliance, ml/mmHg	2.2 ± 0.2	2.0 ± 0.1	1.8 ± 0.2†	1.7 ± 0.2†
Effective arterial elastance, mmHg/ml	1.0 ± 0.0	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1†
Stroke work, mmHg*ml	11,700 ± 592	13,921 ± 695†	14,799 ± 896†	15,941 ± 1,008†
Rate pressure product, AU	6,821 ± 254	7,659 ± 427†	8,217 ± 476†	8,680 ± 325†
Deoxyhemoglobin, μM (n=9)	27 ± 3	48 ± 6†	50 ± 6†	51 ± 6†
HFrEF				
Mean arterial pressure, mmHg	84 ± 3	90 ± 3†	94 ± 3†	100 ± 4†
Systolic arterial pressure, mmHg	117 ± 4	125 ± 5†	129 ± 5†	140 ± 6†
Systolic arterial pressure, ΔmmHg	-	8 ± 2†	12 ± 2*†	23 ± 3*†
Diastolic arterial pressure, mmHg	67 ± 3	73 ± 3†	77 ± 3†	81 ± 3†
Diastolic arterial pressure, ΔmmHg	-	7 ± 1†	9 ± 1*†	13 ± 1*†
Pulse pressure, mmHg	50 ± 3	52 ± 4	53 ± 3*	59 ± 4*†
Heart rate, beats/min	67 ± 2*	68 ± 2*	70 ± 2*	68 ± 2*
Stroke volume, ml/beat	83 ± 4*	81 ± 4*	77 ± 4*	80 ± 4*
Cardiac output, L/min	5.4 ± 0.3	5.5 ± 0.2*	5.3 ± 0.3*	5.4 ± 0.3*
Systemic vascular conductance, ml/min/mmHg	68 ± 4	63 ± 4*†	58 ± 4*†	55 ± 4*†
Systemic vascular resistance, mmHg/L/min	17 ± 2	18 ± 2†	20 ± 2*†	21 ± 3*†
Total arterial compliance, ml/mmHg	1.8 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.5 ± 0.1†
Effective arterial elastance, mmHg/ml	1.4 ± 0.1	1.6 ± 0.2*†	1.7 ± 0.2*†	1.7 ± 0.2*†
Stroke work, mmHg*ml	8,654 ± 464	9,021 ± 472*	8,896 ± 530*	9,982 ± 635*†
Rate pressure product	7,677 ± 310	8,572 ± 469†	8,990 ± 426†	9,445 ± 477†
Deoxyhemoglobin, μM (n=13)	28 ± 2	47 ± 4†	50 ± 4†	53 ± 4†

MVC, maximum voluntary contraction; HFrEF, heart failure with reduced ejection fraction. Data are expressed as means ± SEM. * Significant difference from control, P<0.05; † Significant difference from rest, P<0.05.

CHAPTER 3

HEMODYNAMIC RESPONSES TO SMALL MUSCLE MASS EXERCISE IN HEART FAILURE WITH REDUCED EJECTION FRACTION

Reproduced with permission from Barrett-O'Keefe, Z., Lee, J.F., Berbert, A.,Witman, M.A.H, Nativi-Nicolau, J., Stelhlik, J., Richardson, R.S., and Wray, D.W. (2014). Hemodynamic responses to small muscle mass exercise in heart failure with reduced ejection fraction. Articles in Press. Am J Physiol Heart Circ Physiol (September 26, 2014). doi:10.1152/ajpheart.00527.2014.

Abstract

To better understand the mechanisms responsible for exercise intolerance in heart failure with reduced ejection fraction (HFrEF), the present study sought to evaluate the hemodynamic responses to small muscle mass exercise in this cohort. In 25 HFrEF patients (64 ± 2 yrs) and 17 healthy control subjects of a similar age (64 ± 2 yrs), mean arterial pressure (MAP), cardiac output (CO), and limb blood flow were examined during graded static-intermittent handgrip (HG) and dynamic single-leg knee-extensor (KE) exercise. During HG exercise, MAP increased similarly between groups. CO increased significantly (+1.3 ± 0.3 L/min) in the control group, but remained unchanged across workloads in HFrEF patients. At 15% maximum voluntary contraction (MVC), forearm blood flow was similar between groups, while HFrEF patients exhibited an attenuated increase at the two highest intensities compared to controls, with the greatest difference at the highest workload (352 ± 22 vs. 492 ± 48 ml/min, HFrEF vs. control, 45% MVC). During KE exercise, MAP and CO increased similarly across work rates between groups. However, HFrEF patients exhibited a diminished leg hyperemic response across all work rates, with the most substantial decrement at the highest intensity (1842 ± 64 vs. 2675 ± 81 ml/min, HFrEF vs. control, 15 W). Together, these findings indicate a marked attenuation in exercising limb perfusion attributable to impairments in peripheral vasodilatory capacity during both arm and leg exercise in patients with HFrEF, which likely plays a role in limiting exercise capacity in this patient population.

Introduction

Heart failure with reduced ejection fraction (HFrEF) is associated with debilitating dyspnea and fatigue triggered by exercise, leading to a limited ability to perform everyday tasks and an impaired quality of life (52). Although central cardiac limitations are the paramount characteristic of HFrEF, impaired cardiac function does not fully explain the degree of exercise intolerance and symptom status in this patient population (8, 21, 34, 53, 60). This has steered studies focusing on exercise limitations in HFrEF towards potentially limiting factors in the periphery, with particular emphasis on disease-related changes in the regulation of skeletal muscle blood flow. Initial studies examining the hyperemic response to exercise in the skeletal muscle vasculature of HFrEF patients and healthy individuals utilized cycle ergometry, a large muscle mass exercise paradigm which results in the recruitment of the vast majority of lower limb muscle. These studies observed a marked reduction in blood flow, associated with an attenuation in leg vascular conductance, in HFrEF patients during exercise compared to their healthy counterparts (52, 62-66). However, due to the cardiac limitations associated with HFrEF, the engagement of such a large muscle mass during exercise may have outstripped cardiac pumping capacity (30), likely contributing to the observed attenuation in leg blood flow and vascular conductance in this patient population.

These initial studies utilizing cycle ergometry led to the recognition that skeletal muscle hyperemic and vasodilatory capacity in HFrEF might be better studied by utilizing an exercise paradigm employing a smaller muscle mass, in order to control for cardiac limitations. In an animal model of heart failure, this was accomplished by utilizing an *in situ* spinotrapezius muscle preparation and investigating capillary red blood cell flux, which is indicative of capillary blood flow, during electrically elicited

muscle contractions (46). Results from this study convincingly displayed an attenuation in capillary red blood cell flux in HF animals compared to control animals. In human HF, the two most widely incorporated exercise modalities to study the peripheral hemodynamic responses to exercise while controlling for confounding cardiac limitations are static-intermittent handgrip (HG) and dynamic single-leg knee-extensor (KE) exercise. Despite the implementation of these exercise modalities in a small number of studies, results have not definitively determined the extent of peripheral hemodynamic limitations in this cohort. Indeed, studies using static-intermittent HG exercise have documented both similar (50) and blunted (22, 67) hyperemic responses, linked to impairments in vasodilation, in HFrEF patients compared to healthy individuals, differences likely attributable to the variations in rhythmicity, duration, and intensity of the exercise performed. Using the dynamic single-leg KE exercise model, Esposito *et al.* (14) identified an attenuated hyperemia during maximal effort in HFrEF patients, a response that was likely due to the significantly lower maximal KE work rate in the patient group compared to healthy individuals. In one of the only studies to utilize KE exercise at submaximal work rates in HFrEF patients, Magnusson *et al.* reported similar increases in leg blood flow and vascular conductance between HFrEF and healthy individuals (30), suggesting that impaired peripheral hemodynamics may not contribute to the exercise intolerance in this cohort. Thus, significant controversy remains regarding the HFrEF-associated alteration of peripheral hemodynamics during exercise modalities that minimally challenge central hemodynamics.

It is noteworthy that significant advances in pharmacotherapy have been made since these previous small muscle mass exercise studies were performed (19, 20, 27, 55).

Indeed, the average HFrEF patient now receives a pharmacologic regimen that includes an average of 10 medications, with beta blockers and a host of peripheral vasodilators now included as standard of care (45). Thus, while exercise intolerance remains a key clinical presentation in HFrEF, uncertainty exists regarding the possible role of impaired peripheral hemodynamics in patients who are optimally medicated in this "modern era" of differentiated drug treatment.

Consequently, we sought to systematically examine peripheral responses to small muscle mass exercise in optimally medicated HFrEF patients. To comprehensively assess the hemodynamic response to small muscle mass exercise in HFrEF patients and healthy control subjects of a similar age, we utilized both upper and lower limb exercise paradigms across a wide range of intensities. We hypothesized that compared to healthy controls, HFrEF patients would exhibit an attenuated hyperemic response driven by an impaired vasodilatory capacity during both static-intermittent HG and single-leg KE exercise.

Methods

Subjects

A total of 25 New York Heart Association (NYHA) class II-III HFrEF patients (24 males and 1 female) and 17 healthy controls (16 males and 1 female) of a similar age were recruited to partake in this study either by word of mouth or in the HF clinics at the University of Utah and the Salt Lake City VA Medical Center. All control subjects were nonsmokers, not taking any prescription medication, and were free of overt cardiovascular disease, as indicated by a health history questionnaire. Protocol approval and written informed consent were obtained according to University of Utah and Salt

Lake City Veterans Affairs Medical Center Institutional Review Board requirements. All data collection took place at the Utah Vascular Research Laboratory located at the Veterans Affairs Salt Lake City Geriatric, Research, Education, and Clinical Center. All studies were performed in a thermoneutral environment, with subjects reporting to the laboratory fasted, and not having performed any exercise within 24 hours of the study. Subjects reported to the laboratory on a preliminary day to complete health histories, physical examinations, and perform a graded single-leg knee-extensor test to determine maximal work rate.

Handgrip Exercise

Hemodynamic responses to static-intermittent HG exercise were assessed in 15 control subjects and 23 patients. Subjects rested in the supine position for ≈20 minutes prior to the start of data collection with the right arm abducted at 90°. The elbow joint was extended at heart level to allow subjects to perform HG exercise. First, maximal voluntary contraction (MVC) was established by taking the highest value recorded of 3 maximal contractions using a handgrip dynamometer (Biopac Systems, Goleta, CA). Static-intermittent HG exercise was performed at 3 workloads based on each subject's respective MVC (15, 30, and 45% of MVC). Each exercise level was performed for 3 minutes to ensure the attainment of steady-state hemodynamics. The subjects squeezed the dynamometer to the sound of a metronome at a rate of 1 Hz, and real-time force output was displayed to provide visual feedback to the subjects. A 5 minute recovery period was given between each exercise bout.

Single-Leg Knee-Extensor Exercise

Hemodynamic responses to dynamic single-leg KE exercise were assessed in 16 controls and 16 patients. The KE paradigm implemented in this study has been described in detail previously (3, 4, 7, 26). Briefly, subjects were seated in a semi-recumbent position on an adjustable chair with a cycle ergometer (model 828E; Monark Exercise AB, Vansbro, Sweden) positioned behind them. Resistance was created by applying friction to the flywheel, which was turned by the subject via a metal bar connecting the crank arm of the ergometer to a metal boot in which the subject's foot was placed. Subjects exercised for 3 minutes at 4 work rates (0, 5, 10, and 15 W) while maintaining 60 contractions per minute. A 5 minute recovery period was given between each exercise bout.

Measurements

Ultrasound Doppler assessments

Measurements of brachial and common femoral artery blood velocity and vessel diameter were performed using a Logiq 7 ultrasound Doppler system (GE Medical Systems, Milwaukee, WI) operating in duplex mode. The Logic 7 was equipped with a linear array transducer operating at a Doppler frequency of 5 MHz in high-pulsed repetition frequency mode (2-25 kHz). All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60º or less. The sample volume was maximized according to vessel size and centered within the vessel based on real-time ultrasound visualization. Mean velocity values (angle-corrected, and intensity weighted area under the curve) were automatically calculated using commercially available software (Logic 7). Vessel diameter was obtained during

end diastole (corresponding to an R wave documented by the simultaneous ECG signal; Logic 7) using the same transducer at an imaging frequency ranging from 9 to 14 MHz. The brachial artery of the right arm was insonated approximately midway between the antecubital and axillary regions, medial to the biceps brachii muscle, while the common femoral artery was insonated 2–3 cm proximal to the bifurcation of the common femoral artery into the superficial and deep branches. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area. Analysis of brachial artery diameter was performed using off-line automatic edge-detection brachial analyzer software (Medical Imaging Applications, LLC, Coralville, IA), which is described in detail elsewhere (39). Ultrasound Doppler measurements were performed continuously, with the last 60 seconds of each exercise intensity used for the determination of limb blood flow. Using arterial diameter and V_{mean}, forearm and leg blood flow were calculated as:

$$\textit{Limb blood flow (ml/min)} = (V_{mean} * \pi \textit{ (arterial diameter/2)}^2 * 60)$$

Hemodynamic variables

Stroke volume (SV), arterial blood pressure (ABP), and heart rate (HR) were determined noninvasively. SV was calculated using the Modelflow method which includes age, gender, height, and weight in its algorithm (Beatscope version 1.1; Finapres Medical Systems BV, Amsterdam, The Netherlands) (9), and has been documented to accurately track SV during a variety of experimental protocols including exercise (12, 13, 51). ABP was measured continuously via photoplethysmography (Finometer, Finapres

Medical Systems BV, Amsterdam, The Netherlands), and mean arterial pressure (MAP) was calculated as:

$$MAP\ (mmHg) = diastolic\ arterial\ pressure + (pulse\ pressure * 0.33)$$

Heart rate was monitored from a standard 3 lead electrocardiogram recorded in duplicate on the data acquisition system (Biopac, Goleta, CA) and the Logic 7. Cardiac output (CO) was calculated as:

$$CO\ (L/min) = SV * HR$$

Systemic vascular conductance (SVC) was calculated as:

$$SVC\ (ml/min/mmHg) = CO / MAP$$

Systemic vascular resistance (SVR) was calculated as:

$$SVR\ (mmHg/L/min) = MAP / CO$$

Forearm and leg vascular conductance were calculated as:

$$Limb\ vascular\ conductance\ (ml/min/mmHg) = blood\ flow / MAP$$

Data Analysis

Statistics were performed using commercially available software (SigmaStat 3.10; Systat Software, Point Richmond, CA). For HG exercise, 2x4 repeated measures ANOVA ($\alpha < 0.05$) (group: 2 levels, controls vs. HFrEF) (workload, 4 levels: rest, 15,

30, and 45% of MVC) were performed to determine the hemodynamic responses in controls and HFrEF during exercise of increasing intensity. For KE, 2x5 repeated measures ANOVA ($\alpha < 0.05$) (group: 2 levels, controls vs. HFrEF) (work rate, 5 levels: rest, 0, 5, 10, 15 W) were utilized to determine the hemodynamic responses in age-matched controls and HFrEF during exercise of increasing intensity. The Holm-Sidak method was used for alpha adjustment and post hoc analysis. A Person Product Moment Correlation ($\alpha < 0.05$) was performed to evaluate the association between leg blood flow at 15 W and maximum KE work rate. All group data are expressed as means ± SEM.

Results

Subject Characteristics

Baseline characteristics of the control subjects and HFrEF patients are displayed in **Table 3.1**. Disease-specific characteristics and medications of patients with HFrEF are presented in **Table 3.2**.

Handgrip Exercise

During baseline, prior to HG exercise, there were no significant differences in resting MAP, forearm blood flow, forearm vascular conductance, brachial artery diameter, or CO between groups **(Figure 3.1 and Table 3.3)**. However, resting HR was significantly higher and SV significantly lower in HFrEF patients compared to controls **(Table 3.3)**.

In both groups, MAP and HR increased from resting values in an exercise intensity-dependent manner, with no difference between groups **(Figure 3.1 and Table 3.3)**. CO increased from rest at all exercise intensities in the control group, but remained

unchanged in the HFrEF patients **(Table 3.3)**. This significant difference in the CO response in HFrEF patients appears to be the result of a tendency for decreased SV across workloads, though this reduction did not reach statistical significance (**Table 3.3**). During HG exercise, SVC decreased and SVR increased in HFrEF patients, but remained unchanged in the control group (**Table 3.3)**.

At the lowest intensity (15% MVC), forearm blood flow and vascular conductance increased to a similar degree between groups; however, at the higher workloads (30 and 45% MVC), HFrEF patients exhibited significantly lower forearm blood flow (30 and 45% MVC) and vascular conductance (45% MVC) compared to controls **(Figure 3.1 and Table 3.3)**.

Single-Leg Knee-Extensor Exercise

During the baseline period prior to KE exercise, there were no significant differences in any indices of central or peripheral hemodynamics between groups **(Figure 3.2 and Table 3.4)**. In both groups, MAP and CO increased from resting values in an exercise intensity-dependent manner, with no differences between groups **(Figure 3. 2 and Table3. 4)**. HR also increased in an intensity-dependent manner, with a significantly elevated HR in HFrEF patients compared to controls (5, 10, and 15W) (**Table 3.4**). SV, SVC, SVR remained unchanged in both groups across all KE exercise intensities (**Table 3.4**). In contrast to HG exercise, both leg blood flow and leg vascular conductance were markedly reduced at all exercise intensities in HFrEF patients compared to controls (**Figure 3.2 and Table 3.4**), with the greatest reduction (25-35%) present at the highest exercise intensity (15 W). Additionally, there was a strong positive association, between

leg blood flow at 15 W and the maximum KE work rate in HFrEF which was not apparent in the control group **(Figure 3.3)**.

Discussion

This study sought to comprehensively evaluate the hemodynamic responses induced by limb-specific, small muscle mass exercise across a wide range of exercise intensities in HFrEF patients and healthy, age-matched controls. During HG exercise, a divergent hemodynamic response was observed across HG intensities in the HFrEF group. Specifically, both groups exhibited a similar forearm hyperemic and vasodilatory response during lower intensity (15% MVC) HG exercise, but HFrEF patients exhibited a 15-25% attenuation in forearm blood flow at higher intensities (30 and 45% MVC), due to an impaired vasodilatory capacity. During KE exercise, HFrEF patients exhibited a 20-35% lower leg blood flow and vascular conductance during KE exercise compared to control subjects, with the most substantial decrements at the highest exercise intensity (15 W). Together, these findings indicate that HFrEF patients on modern, optimized pharmacotherapy exhibit a severely compromised ability to vasodilate vasculature of both the upper and lower limbs, thus restricting perfusion of the exercising skeletal muscle and likely limiting exercise capacity in this patient group.

Regulation of Skeletal Muscle Blood Flow during Exercise in HFrEF

A hallmark symptom of patients with HFrEF is an impaired exercise tolerance and an associated reduction in maximal exercise capacity (16, 40, 60, 65). Interestingly, the degree of left ventricular dysfunction does not fully explain the degree of exercise intolerance or symptom status in this patient group (8, 21, 34, 53, 60), supporting the

possibility that peripheral hemodynamic dysfunction may contribute to exercise intolerance. Evidence from previous studies (62-66) indicate a functional role for impaired peripheral hemodynamics in limiting exercise capacity in this cohort during peak cycling exercise, where an apparent reduction in leg vascular conductance was observed in patients with HFrEF compared to healthy individuals, contributing to the documented impairment in perfusion. However, the use of a large muscle mass exercise paradigm presents limitations in distinguishing abnormalities in peripheral hemodynamics in HFrEF. During exercise that recruits a large fraction of total body muscle mass, central circulatory factors play an increasingly important role in the preservation of MAP (41, 47, 49). Due to impaired left ventricular function during whole body exercise, patients with HFrEF largely depend on systemic vasoconstriction (i.e. reductions in SVC) in order to maintain MAP (52). This may indicate that the markedly lower leg vascular conductance and associated decrement in perfusion of the exercising skeletal muscle during cycling exercise might solely be due to the maintenance of MAP versus limitations in vasodilatory capacity in this patient group (52, 66).

In order to investigate how altering the total amount of muscle recruited during exercise might affect the peripheral hemodynamic responses to exercise in this patient group, LeJemtel *et al.* (28) examined the differences in leg blood flow during maximal single- and double-legged upright cycling in HFrEF patients and healthy control subjects. Interestingly, they observed similar leg blood flow values in the HFrEF patients during both single-and double-legged exercise modalities, which they attributed to an impaired vasodilator response in the patient group (28). These results were indirectly supported by Jondeau *et al*. (23), who utilized maximal arm cycling exercise superimposed on maximal

leg cycling to examine the impact of HF on cardiopulmonary reserve. In this study, addition of arm exercise while cycling provoked an increase in peak oxygen (O_2) consumption in severe HF patients, but not in healthy controls, which was interpreted as evidence for an inadequate vasodilator response to exercise resulting in impaired O_2 delivery, as the arterial-venous O_2 difference is near maximal in HFrEF during maximal leg cycling (25).

While these novel studies were some of the first to investigate the hemodynamic response to exercise which recruited differing amounts of muscle mass during all combinations of arm and leg cycling, it is likely that some degree of cardiac limitation still confound these results. This is even the case during maximum single-legged cycling, where Martin *et al.* (31) has documented that the CO achieved in HFrEF patients is similar compared CO values during maximum double-legged cycling in this patient group. Thus, in the present study, we employed two limb-specific small muscle mass exercise modalities (static-intermittent HG and dynamic single-leg KE exercise) across a range of submaximal exercise intensities in order to more thoroughly investigate the peripheral hemodynamic response to exercise in HFrEF in isolation of the significant confounding effects of CO limitations and MAP regulation imposed by large muscle mass exercise.

Hemodynamic Responses to Handgrip Exercise

Due to the accessibility and limited cardiorespiratory stress associated with this modality, handgrip exercise has been utilized in a number of studies over the past several decades to investigate the peripheral hemodynamic response in HFrEF patients. Indeed, Zelis *et al.* (67) investigated the peripheral blood flow response to graded static-

intermittent handgrip exercise in HFrEF patients compared to control subjects in the early 1970's, and documented an impaired exercise hyperemia in this patient group. However, it is noteworthy that this study utilized venous plethysmography to measure blood flow during the 10 second relaxation phase between 5 second isometric contractions, an approach that precludes assessment of the phasic pattern of blood flow associated with rhythmic handgrip exercise. Thus, while this seminal work was among the first to examine disease-related changes in regional blood flow during small muscle mass exercise, the inherent limitations associated with plethysmographic determination of blood flow (29, 67) during low-cadence isometric handgrip exercise left some uncertainty regarding the true nature of the hyperemic response in this patient group.

In the current study, we utilized Doppler ultrasound to measure blood flow continuously during 1 Hz static-intermittent handgrip exercise of graded intensity in HFrEF patients and control subjects of a similar age. Interestingly, the HFrEF patients exhibited a divergent hyperemic and vasodilatory response to static-intermittent HG exercise. Specifically, we observed comparable changes in forearm blood flow and vascular conductance during low intensity (15% MVC) exercise between HFrEF patients and healthy controls, and a divergence at higher intensities, with HFrEF patients exhibiting an impaired ability to alter vasomotor tone and therefore increase blood flow **(Figure 3. 1 and Table 3.3)**. Using a similar HG exercise model and a single, low-intensity workload (4.4kg, ≈15% MVC), Shoemaker *et al.* (50) reported a similar hemodynamic response in HFrEF patients and controls, suggesting a preserved vasomotor regulation and hyperemic response in HFrEF patients when performing HG exercise. However, when viewed in the context of the current findings across a wide

range of exercise intensities (**Figure 3.1**), this former study did not characterize the full scope of the hemodynamic and vasodilatory response in HFrEF. Thus, using beat-to-beat measurements, a dynamic (1 Hz) exercise cadence, and a wide range of exercise intensities, the present study both confirms and extends these previous findings, unmasking a marked impairment in forearm blood flow that may be attributed to a limited limb-specific vasodilatory capacity in the exercising skeletal muscle vasculature of HFrEF patients.

This reduction in forearm blood flow and vascular conductance in HFrEF patients was accompanied by a clear lack of an increase in CO across increasing intensities of exercise in this patient group **(Table 3.3)**, which is in contrast to the robust (≈1 L/min, **Table 3.3**) increase observed in the control group at the highest exercise intensity. While the mechanisms responsible for this physiological adjustment to static-intermittent HG in HFrEF are unknown, a potential explanation for the absence of an exercise-induced increase in CO is the substantial reduction in SVC exhibited in this patient group **(Table 3.3)**. Indeed, SVR (the inverse of SVC), is commonly used as a measurement of nonpulsatile arterial load on the left ventricle (35, 37, 59) and it is well-established that patients with HFrEF are afterload-sensitive (6, 24, 48) and face impairments in left ventricular systolic function if arterial afterload is increased (24). However, it is a possibility that the unique cardiovascular adjustments associated with HG exercise in HFrEF might not elicit a sufficient stimulus to induce an increase in CO, thus requiring a reduction SVC in order to increase MAP in this patient group. Thus, it is tempting to speculate that during HG exercise, impaired blood flow within the exercising muscle vasculature may have indirectly been limited by the inappropriate exercise-induced

increase in CO in HFrEF patients. The “real world” implications of this response are not trivial; indeed, the cardiovascular adjustments observed with this HG exercise modality may also be present during tasks of daily living that utilize the upper limbs, such as carrying groceries. Thus, the observed impairment in vasodilation and the accompanying absence of central responses may be viewed as representing a previously unexplored aspect of exercise intolerance that could contribute to the diminished quality of life in this patient group.

Hemodynamic Responses to Single-Leg Knee-Extensor Exercise

While these data during HG exercise demonstrate a clear impairment in forearm hemodynamics, exercise intolerance in HFrEF patients has classically been documented during tasks primarily involving locomotion (16, 40, 60, 65), and thus further investigating the hemodynamic responses to physical exertion in the skeletal muscle vasculature of the legs is also warranted. During single-leg KE exercise, HFrEF patients exhibited a persistent impairment in the ability to overcome the tonic vasomotor restraint of the lower limb vasculature across increasing exercise intensities **(Table 3.4)**, thus limiting perfusion of the exercising limb (**Figure 3.2**). These results are in contrast to Magnusson *et al.* (30), one of the only other studies to examine changes leg blood flow during submaximal KE exercise in this patient group. In this previous study, similar increases in leg blood flow and vascular conductance were observed across graded exercise intensities in HFrEF patients compared to controls. Though the reasons for this discrepancy between this former study and the present findings are not immediately obvious, one likely explanation is the evolution of pharmacotherapy associated with the treatment of HFrEF. Indeed, while a host of new drug classes have been developed and

proven efficacious in the treatment of HFrEF since this previous study was undertaken, one noteworthy change in pharmacologic standard of care for these patients over the past two decades is a reduction in the prevalence of positive inotropes. Indeed, the vast majority of patients in the aforementioned study were prescribed digoxin, a drug that has been documented to attenuate sympathetic nervous system activity (58), reduce circulating norepinephrine (2, 17, 54), and increase peripheral artery vasodilation (32) in HFrEF patients. This supports the concept that the higher exercising leg blood flow documented by Magnusson *et al.* might be due to a digoxin-induced release of sympathetic restraint on peripheral vascular tone.

Additionally, there was a strong association between leg blood flow at a given absolute work rate (15 W) and the maximum KE work rate achieved in HFrEF patients which was not present in the control group **(Figure 3.3)**. This is a unique finding, as blood flow is traditionally thought to match the metabolic demand of a given amount of work performed (44). Indeed, the current study **(Figure 3.3)** and previous work (4), have documented similar leg blood flow values during KE exercise of a given absolute work rate in healthy individuals with varying maximal knee-extensor work rates. This HFrEF-specific relationship suggests that the more severe the impairment in exercise hyperemia, the more substantial the decrements in these patients' respective capacity to perform work.

In contrast to HG exercise, the exercise-induced changes in CO during KE exercise in the present study were remarkably similar between groups (**Table 3.4**), a differing response that is potentially attributed to a preserved SVC during KE exercise in HFrEF **(Table 3.4)**. This comparable central response serves to highlight the advantage

of the KE exercise model, a small muscle mass modality that is capable of eliciting large, concomitant linear increases in skeletal muscle blood flow and CO without potential confounding factors limiting the intensity-dependent increase in CO capacity performed at submaximal intensities. Indeed, at the highest exercise intensity (15 W), almost identical CO values were observed in HFrEF and control groups, while leg blood flow was ≈35% lower in the patient group. Together, these central and peripheral responses provide new evidence for a persistent restraint of skeletal muscle blood flow during leg exercise in optimally medicated HFrEF patients that cannot be explained by disease-related impairments in CO, implicating the lower limb peripheral vasculature as a significant contributor to the limited exercise capacity displayed by optimally medicated patients with HFrEF.

Perspectives

It is important to acknowledge that O_2 transport during exercise is a multifaceted process which includes both the bulk delivery of O_2 to the exercising muscle (i.e convective O_2 transport) via increases in arterial blood flow as well as the local O_2 distribution to the exercising skeletal muscle via the unloading of O_2 from hemoglobin to the skeletal muscle mitochondria (i.e. diffusional O_2 conductance) (42, 43, 56, 57). While the present study convincingly demonstrates an attenuated blood flow response during graded exercise in HFrEF patients, likely contributing to an impairment in convective O_2 transport, deficiencies in diffusional conductance might be contributing to a systemic impairment in O_2 transport in patients with HFrEF during exercise as well. Indeed, Esposito *et al.(14)* demonstrated an attenuated diffusional O_2 conductance in HFrEF patients compared to control subjects during maximal KE exercise, though this response

may have been attributable to the significantly lower maximal KE work rate in the patient group. Further studies are certainly warranted to investigate whether the impairments in diffusional conductance present in HFrEF during maximal exercise extends to submaximal exercise intensities.

It is widely accepted that one of the goals of pharmacotherapy for patients with HFrEF has been to relieve symptoms, many of which are associated with physical exertion and exercise intolerance (5, 10). While the early historical progression of the optimization of pharmacological therapy in HFrEF largely focused on improving left ventricular function through the use of positive inotropes (5, 18), the introduction of vasodilator therapy in the 1980's was a therapeutic milestone (5, 10). Indeed, the demonstrated beneficial effect of antihypertensive drugs including nitrate-based medications (i.e. hydralazine) (11) and angiotensin-converting enzyme (ACE) inhibitors (1, 11, 15) on exercise intolerance and exercise-related symptoms has established important pleiotropic properties for these drug classes that are of significant value in the treatment of HFrEF. However, based on the findings from the current study, the combined effect of vasodilators currently available and widely prescribed in HFrEF (i.e. ACE inhibitors, aldosterone receptor antagonists, angiotensin receptor blockers, and vasodilating beta-adrenergic antagonists such as carvedilol) might not be sufficient to restore peripheral vasodilation during exercise.

Experimental Considerations

Several limitations to the present study are worth noting. While skeletal muscle vasodilation contributes significantly to the overall regulation of exercise hyperemia, we recognize that decrements in vasodilatory capacity are not the sole factor responsible for

the observed impairment in blood flow during exercise in HFrEF. Indeed, disease-related changes in vascular architecture, including capillary rarefaction (36, 61) and impairments in vascular flow capacity (33) may contribute to reduced limb blood flow independent of disease-related alterations in vascular control. We also recognize that the observed reduction in leg blood flow in HFrEF patients in the face of similar CO values between groups raises the question of whether blood flow distribution may be disturbed in the HFrEF cohort. While the current study cannot answer this question, we acknowledge the possibility that increased blood flow to respiratory muscles may be partially responsible for our observed results during exercise in HFrEF. Indeed, during cycling exercise, Olson *et al.* (38) documented that HFrEF patients preferentially "steal" blood flow from the exercising skeletal muscle in order to accommodate their enhanced work of breathing. Additionally, in the present study, calculations of both systemic and regional vascular conductance were made using only arterial pressure, rather than the more conventional arterial-venous pressure difference. Thus, we cannot exclude the possibility that potential changes in venous pressure associated with HFrEF may have confounded our findings. However, we have recently reported similar venous pressures during maximal KE exercise in HFrEF and control subjects (14), an observation that somewhat mitigates this concern. We also acknowledge the lack of a direct assessment of exercise tolerance in the HFrEF group, though the observation that leg blood flow at a given absolute work rate was strongly associated with the maximum KE work rate in HFrEF patients (and not in healthy controls) is a strong indication that impairments in exercise hyperemia contributed to the limited work capacity in the patient group. Finally, based on the differences in which workloads were determined between handgrip and KE exercise, we

wish to emphasize that a direct comparison of hemodynamic responses between these two exercise modalities is not possible.

Conclusions

Using a wide range of exercise intensities, we have identified a clear impairment in perfusion of both the upper and lower limbs during small muscle mass exercise in HFrEF patients. These findings indicate that HFrEF patients on modern, optimized pharmacotherapy exhibit a severely compromised peripheral hyperemic response, implicating maladaptations in the peripheral vasculature and its regulation as potential factors contributing to the reduced exercise capacity in this patient group.

References

1. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). The CONSENSUS Trial Study Group. *N Engl J Med* 316: 1429-1435, 1987.

2. **Alicandri C, Fariello R, Boni E, Zaninelli A, Castellano M, Beschi M, Agabiti Rosei E, and Muiesan G**. Captopril versus digoxin in mild-moderate chronic heart failure: a crossover study. *J Cardiovasc Pharmacol* 9 Suppl 2: S61-67, 1987.

3. **Andersen P, Adams RP, Sjogaard G, Thorboe A, and Saltin B**. Dynamic knee extension as model for study of isolated exercising muscle in humans. *J Appl Physiol (1985)* 59: 1647-1653, 1985.

4. **Andersen P, and Saltin B**. Maximal perfusion of skeletal muscle in man. *J Physiol* 366: 233-249, 1985.

5. **Armstrong PW, and Moe GW**. Medical advances in the treatment of congestive heart failure. *Circulation* 88: 2941-2952, 1993.

6. **Asanoi H, Sasayama S, and Kameyama T**. Ventriculoarterial coupling in normal and failing heart in humans. *Circ Res* 65: 483-493, 1989.

7. **Barrett-O'Keefe Z, Ives SJ, Trinity JD, Morgan G, Rossman MJ, Donato AJ, Runnels S, Morgan DE, Gmelch BS, Bledsoe AD, Richardson RS, and Wray DW**. Taming the "sleeping giant": the role of endothelin-1 in the regulation of skeletal muscle blood flow and arterial blood pressure during exercise. *Am J Physiol Heart Circ Physiol* 304: H162-169, 2013.

8. **Benge W, Litchfield RL, and Marcus ML**. Exercise capacity in patients with severe left ventricular dysfunction. *Circulation* 61: 955-959, 1980.

9. **Bogert LW, and van Lieshout JJ**. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Exp Physiol* 90: 437-446, 2005.

10. **Cohn JN**. The management of chronic heart failure. *N Engl J Med* 335: 490-498, 1996.

11. **Cohn JN, Johnson G, Ziesche S, Cobb F, Francis G, Tristani F, Smith R, Dunkman WB, Loeb H, Wong M, and et al.** A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. *N Engl J Med* 325: 303-310, 1991.

12. **de Vaal JB, de Wilde RB, van den Berg PC, Schreuder JJ, and Jansen JR**. Less invasive determination of cardiac output from the arterial pressure by aortic diameter-calibrated pulse contour. *Br J Anaesth* 95: 326-331, 2005.

13. **de Wilde RB, Geerts BF, Cui J, van den Berg PC, and Jansen JR**. Performance of three minimally invasive cardiac output monitoring systems. *Anaesthesia* 64: 762-769, 2009.

14. **Esposito F, Mathieu-Costello O, Shabetai R, Wagner PD, and Richardson RS**. Limited maximal exercise capacity in patients with chronic heart failure: partitioning the contributors. *J Am Coll Cardiol* 55: 1945-1954, 2010.

15. **Fletcher RD, Cintron GB, Johnson G, Orndorff J, Carson P, and Cohn JN**. Enalapril decreases prevalence of ventricular tachycardia in patients with chronic congestive heart failure. The V-HeFT II VA Cooperative Studies Group. *Circulation* 87: VI49-55, 1993.

16. **Franciosa JA, Ziesche S, and Wilen M**. Functional capacity of patients with chronic left ventricular failure. Relationship of bicycle exercise performance to clinical and hemodynamic characterization. *Am J Med* 67: 460-466, 1979.

17. **Gheorghiade M, Hall V, Lakier JB, and Goldstein S**. Comparative hemodynamic and neurohormonal effects of intravenous captopril and digoxin and their combinations in patients with severe heart failure. *J Am Coll Cardiol* 13: 134-142, 1989.

18. **Goldhaber JI, and Hamilton MA**. Role of inotropic agents in the treatment of heart failure. *Circulation* 121: 1655-1660, 2010.

19. **Gottlieb SS, McCarter RJ, and Vogel RA**. Effect of beta-blockade on mortality among high-risk and low-risk patients after myocardial infarction. *N Engl J Med* 339: 489-497, 1998.

20. **Heidenreich PA, Lee TT, and Massie BM**. Effect of beta-blockade on mortality in patients with heart failure: a meta-analysis of randomized clinical trials. *J Am Coll Cardiol* 30: 27-34, 1997.

21. **Higginbotham MB, Morris KG, Conn EH, Coleman RE, and Cobb FR**. Determinants of variable exercise performance among patients with severe left ventricular dysfunction. *Am J Cardiol* 51: 52-60, 1983.

22. **Imaizumi T, Hirooka Y, and Takeshita A**. Decreased skeletal muscle vasodilation in patients with congestive heart failure. *Jpn Circ J* 56: 500-503, 1992.

23. **Jondeau G, Katz SD, Zohman L, Goldberger M, McCarthy M, Bourdarias JP, and LeJemtel TH**. Active skeletal muscle mass and cardiopulmonary reserve. Failure to attain peak aerobic capacity during maximal bicycle exercise in patients with severe congestive heart failure. *Circulation* 86: 1351-1356, 1992.

24. **Kameyama T, Asanoi H, Ishizaka S, and Sasayama S**. Ventricular load optimization by unloading therapy in patients with heart failure. *J Am Coll Cardiol* 17: 199-207, 1991.

25. **Katz SD, Maskin C, Jondeau G, Cocke T, Berkowitz R, and LeJemtel T**. Near-maximal fractional oxygen extraction by active skeletal muscle in patients with chronic heart failure. *J Appl Physiol (1985)* 88: 2138-2142, 2000.

26. **Lawrenson L, Poole JG, Kim J, Brown C, Patel P, and Richardson RS**. Vascular and metabolic response to isolated small muscle mass exercise: effect of age. *Am J Physiol Heart Circ Physiol* 285: H1023-1031, 2003.

27. **Lechat P, Packer M, Chalon S, Cucherat M, Arab T, and Boissel JP**. Clinical effects of beta-adrenergic blockade in chronic heart failure: a meta-analysis of double-blind, placebo-controlled, randomized trials. *Circulation* 98: 1184-1191, 1998.

28. **LeJemtel TH, Maskin CS, Lucido D, and Chadwick BJ**. Failure to augment maximal limb blood flow in response to one-leg versus two-leg exercise in patients with severe heart failure. *Circulation* 74: 245-251, 1986.

29. **Longhurst J, Capone RJ, Mason DT, and Zelis R**. Comparison of blood flow measured by plethysmograph and flowmeter during steady state forearm exercise. *Circulation* 49: 535-540, 1974.

30. **Magnusson G, Kaijser L, Sylven C, Karlberg KE, Isberg B, and Saltin B**. Peak skeletal muscle perfusion is maintained in patients with chronic heart failure when only a small muscle mass is exercised. *Cardiovasc Res* 33: 297-306, 1997.

31. **Martin WH, 3rd, Berman WI, Buckey JC, Snell PG, and Blomqvist CG**. Effects of active muscle mass size on cardiopulmonary responses to exercise in congestive heart failure. *J Am Coll Cardiol* 14: 683-694, 1989.

32. **Mason DT, and Braunwald E**. Studies on Digitalis. X. Effects of Ouabain on Forearm Vascular Resistance and Venous Tone in Normal Subjects and in Patients in Heart Failure. *J Clin Invest* 43: 532-543, 1964.

33. **McAllister RM, Laughlin MH, and Musch TI**. Effects of chronic heart failure on skeletal muscle vascular transport capacity of rats. *Am J Physiol* 264: H689-691, 1993.

34. **Metra M, Raddino R, Dei Cas L, and Visioli O**. Assessment of peak oxygen consumption, lactate and ventilatory thresholds and correlation with resting and exercise hemodynamic data in chronic congestive heart failure. *Am J Cardiol* 65: 1127-1133, 1990.

35. **Nichols WW, and Pepine CJ**. Left ventricular afterload and aortic input impedance: implications of pulsatile blood flow. *Prog Cardiovasc Dis* 24: 293-306, 1982.

36. **Nusz DJ, White DC, Dai Q, Pippen AM, Thompson MA, Walton GB, Parsa CJ, Koch WJ, and Annex BH**. Vascular rarefaction in peripheral skeletal muscle after experimental heart failure. *Am J Physiol Heart Circ Physiol* 285: H1554-1562, 2003.

37. **O'Rourke MF**. Vascular impedance in studies of arterial and cardiac function. *Physiol Rev* 62: 570-623, 1982.

38. **Olson TP, Joyner MJ, Dietz NM, Eisenach JH, Curry TB, and Johnson BD**. Effects of respiratory muscle work on blood flow distribution during exercise in heart failure. *J Physiol* 588: 2487-2501, 2010.

39. **Padilla J, Johnson BD, Newcomer SC, Wilhite DP, Mickleborough TD, Fly AD, Mather KJ, and Wallace JP**. Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus. *Cardiovasc Ultrasound* 6: 44, 2008.

40. **Patterson JA, Naughton J, Pietras RJ, and Gunnar RM**. Treadmill exercise in assessment of the functional capacity of patients with cardiac disease. *Am J Cardiol* 30: 757-762, 1972.

41. **Pawelczyk JA, Hanel B, Pawelczyk RA, Warberg J, and Secher NH**. Leg vasoconstriction during dynamic exercise with reduced cardiac output. *J Appl Physiol* 73: 1838-1846, 1992.

42. **Poole DC, Hirai DM, Copp SW, and Musch TI**. Muscle oxygen transport and utilization in heart failure: implications for exercise (in)tolerance. *Am J Physiol Heart Circ Physiol* 302: H1050-1063, 2012.

43. **Richardson RS**. Oxygen transport and utilization: an integration of the muscle systems. *Adv Physiol Educ* 27: 183-191, 2003.

44. **Richardson RS, Harms CA, Grassi B, and Hepple RT**. Skeletal muscle: master or slave of the cardiovascular system? *Med Sci Sports Exerc* 32: 89-93, 2000.

45. **Richardson RS, and Saltin B**. Human muscle blood flow and metabolism studied in the isolated quadriceps muscles. *Med Sci Sports Exerc* 30: 28-33, 1998.

46. **Richardson TE, Kindig CA, Musch TI, and Poole DC**. Effects of chronic heart failure on skeletal muscle capillary hemodynamics at rest and during contractions. *J Appl Physiol (1985)* 95: 1055-1062, 2003.

47. **Saltin B**. Hemodynamic adaptations to exercise. *Am J Cardiol* 55: 42D-47D, 1985.

48. **Schwartzenberg S, Redfield MM, From AM, Sorajja P, Nishimura RA, and Borlaug BA**. Effects of vasodilation in heart failure with preserved or reduced ejection fraction implications of distinct pathophysiologies on response to therapy. *J Am Coll Cardiol* 59: 442-451, 2012.

49. **Secher NH, Clausen JP, Klausen K, Noer I, and Trap-Jensen J**. Central and regional circulatory effects of adding arm exercise to leg exercise. *Acta Physiol Scand* 100: 288-297, 1977.

50. **Shoemaker JK, Naylor HL, Hogeman CS, and Sinoway LI**. Blood flow dynamics in heart failure. *Circulation* 99: 3002-3008, 1999.

51. **Sugawara J, Tanabe T, Miyachi M, Yamamoto K, Takahashi K, Iemitsu M, Otsuki T, Homma S, Maeda S, Ajisaka R, and Matsuda M**. Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between Modelflow method and Doppler echocardiography method. *Acta Physiol Scand* 179: 361-366, 2003.

52. **Sullivan MJ, and Hawthorne MH**. Exercise intolerance in patients with chronic heart failure. *Prog Cardiovasc Dis* 38: 1-22, 1995.

53. **Szlachcic J, Massie BM, Kramer BL, Topic N, and Tubau J**. Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. *Am J Cardiol* 55: 1037-1042, 1985.

54. **Tsakiris AG, Donald DE, Sturm RE, and Wood EH**. Volume, ejection fraction, and internal dimensions of left ventricle determined by biplane videometry. *Fed Proc* 28: 1358-1367, 1969.

55. **van der Harst P, and de Boer RA**. Statins in the treatment of heart failure. *Circ Heart Fail* 3: 462-464, 2010.

56. **Wagner PD**. Determinants of maximal oxygen transport and utilization. *Annu Rev Physiol* 58: 21-50, 1996.

57. **Wagner PD**. Systemic oxygen transport and utilization. *J Breath Res* 2: 024001, 2008.

58. **Watanabe AM**. Digitalis and the autonomic nervous system. *J Am Coll Cardiol* 5: 35A-42A, 1985.

59. **Weber KT, Janicki JS, Hunter WC, Shroff S, Pearlman ES, and Fishman AP**. The contractile behavior of the heart and its functional coupling to the circulation. *Prog Cardiovasc Dis* 24: 375-400, 1982.

60. **Weber KT, Kinasewitz GT, Janicki JS, and Fishman AP**. Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. *Circulation* 65: 1213-1223, 1982.

61. **Williams AD, Selig S, Hare DL, Hayes A, Krum H, Patterson J, Geerling RH, Toia D, and Carey MF**. Reduced exercise tolerance in CHF may be related to factors other than impaired skeletal muscle oxidative capacity. *J Card Fail* 10: 141-148, 2004.

62. **Wilson JR, and Ferraro N**. Effect of the renin-angiotensin system on limb circulation and metabolism during exercise in patients with heart failure. *J Am Coll Cardiol* 6: 556-563, 1985.

63. **Wilson JR, Ferraro N, and Wiener DH**. Effect of the sympathetic nervous system on limb circulation and metabolism during exercise in patients with heart failure. *Circulation* 72: 72-81, 1985.

64. **Wilson JR, Martin JL, and Ferraro N**. Impaired skeletal muscle nutritive flow during exercise in patients with congestive heart failure: role of cardiac pump dysfunction as determined by the effect of dobutamine. *Am J Cardiol* 53: 1308-1315, 1984.

65. **Wilson JR, Martin JL, Ferraro N, and Weber KT**. Effect of hydralazine on perfusion and metabolism in the leg during upright bicycle exercise in patients with heart failure. *Circulation* 68: 425-432, 1983.

66. **Wilson JR, Martin JL, Schwartz D, and Ferraro N**. Exercise intolerance in patients with chronic heart failure: role of impaired nutritive flow to skeletal muscle. *Circulation* 69: 1079-1087, 1984.

67. **Zelis R, Longhurst J, Capone RJ, and Mason DT**. A comparison of regional blood flow and oxygen utilization during dynamic forearm exercise in normal subjects and patients with congestive heart failure. *Circulation* 50: 137-143, 1974.

Figure 3.1 Forearm blood flow (*top*) and mean arterial pressure (*bottom*) at rest and during static-intermittent handgrip exercise in control subjects and heart failure patients with reduced ejection fraction (HFrEF). * Significant difference from control, $P<0.05$; † Significant difference from rest, $P<0.05$.

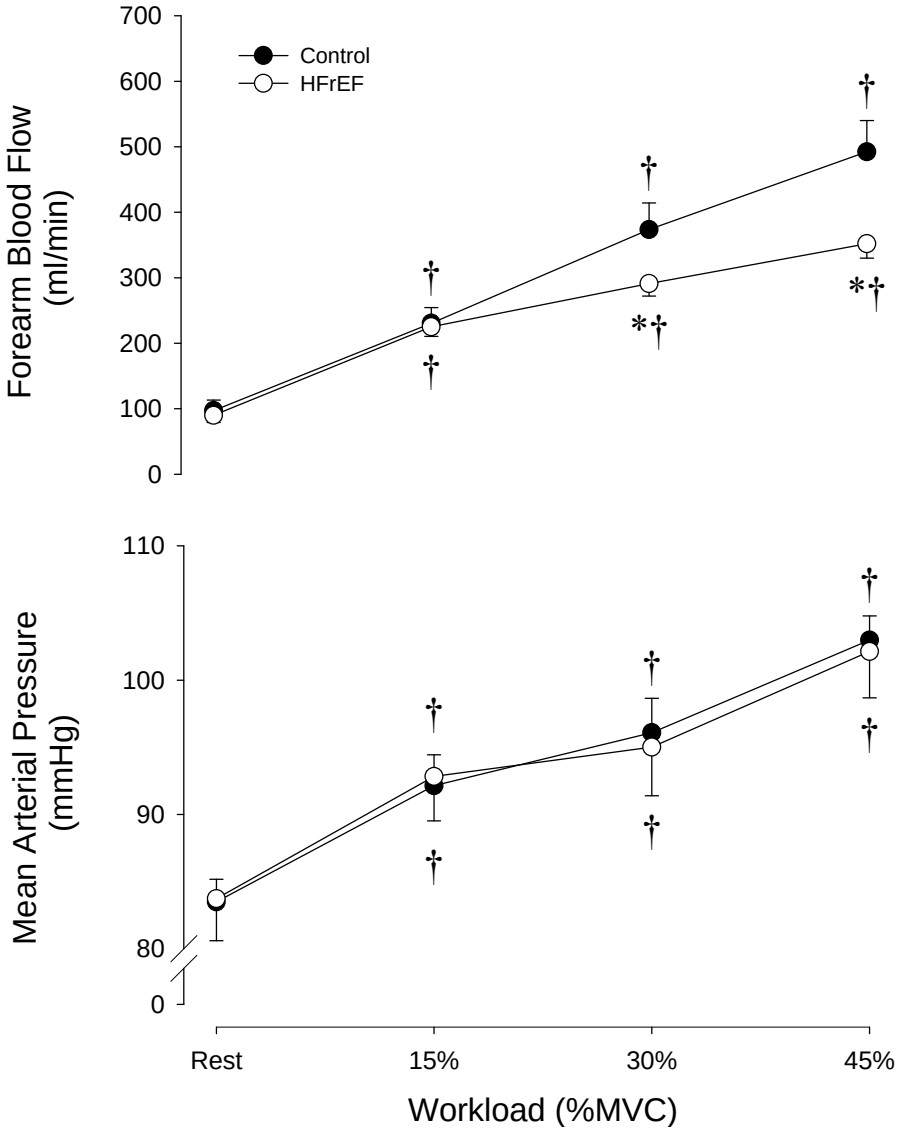

Forearm Blood Flow
(ml/min)
700
600
500
400
300
200
100
0
Control
HFrEF
Mean Arterial Pressure
(mmHg)
110
100
90
80
0
Rest
15%
30%
45%
Workload (%MVC)

Figure 3.2 Leg blood flow (*top*) and mean arterial pressure (*bottom*) at rest and during dynamic single-leg knee-extensor exercise in control subjects and heart failure patients with reduced ejection fraction (HFrEF). * Significant difference from control, $P<0.05$; † Significant difference from rest, $P<0.05$.

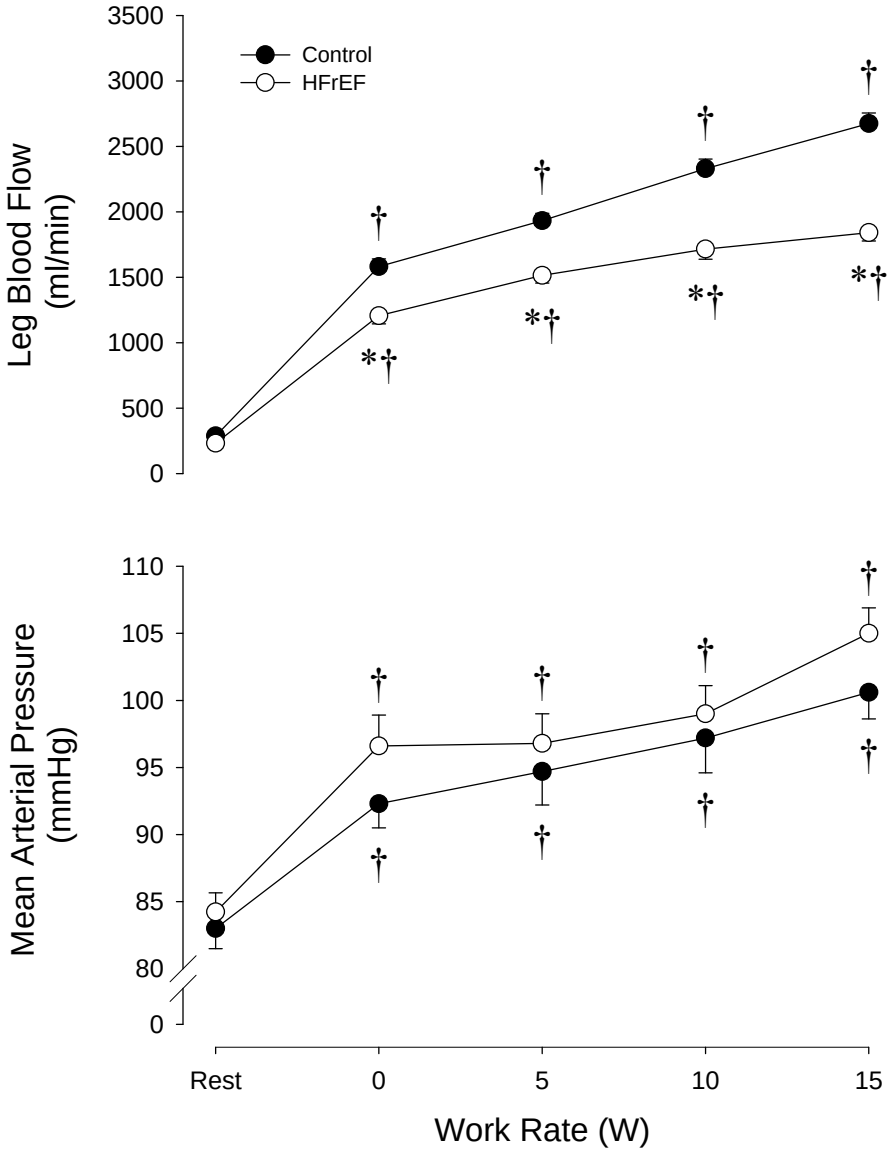

Leg Blood Flow (ml/min)
3500
3000
2500
2000
1500
1000
500
0
Control
HFrEF
Mean Arterial Pressure (mmHg)
110
105
100
95
90
85
80
0
Rest
0
5
10
15
Work Rate (W)

Figure 3.3 Relationship between leg blood flow at 15 W and maximal single-leg knee-extensor work rate in control subjects and heart failure patients with reduced ejection fraction (HFrEF). Significant Pearson Product Moment Correlation coefficient, $P<0.05$.

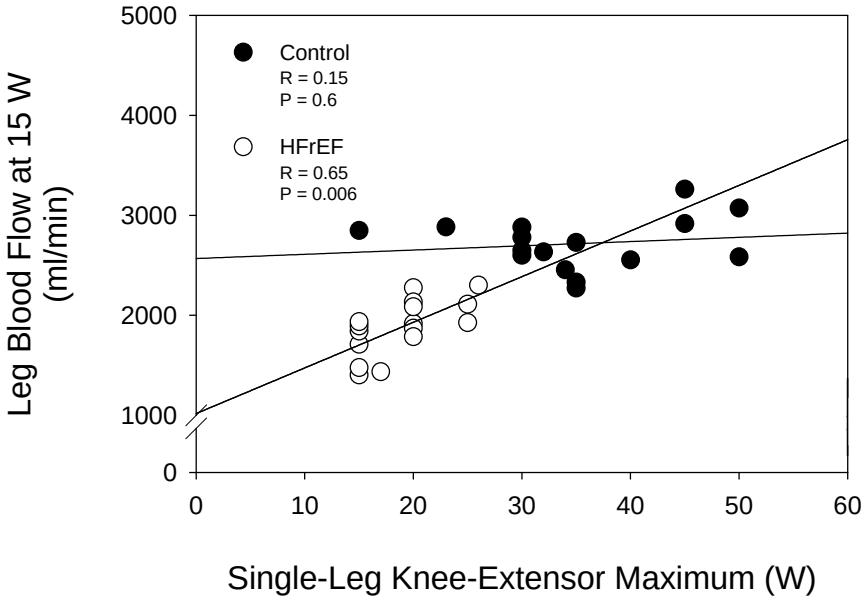

Leg Blood Flow at 15 W
(ml/min)
5000
4000
3000
2000
1000
0
Control
R = 0.15
P = 0.6
HFrEF
R = 0.65
P = 0.006
0
10
20
30
40
50
60
Single-Leg Knee-Extensor Maximum (W)

Table 3.1 *Subject characteristics*

	Control (n = 17)	HFrEF (n = 25)
Age, yrs	64 ± 2	64 ± 2
Height, cm	177 ± 2	171 ± 3
Weight, kg	87 ± 7	85 ± 3
Body mass index, kg/m^2	25 ± 1	28 ± 1
Systolic blood pressure, mmHg	120 ± 3	117 ± 4
Diastolic blood pressure, mmHg	70 ± 2	67 ± 3
Maximum voluntary contraction, kg	27 ± 2 (n = 15)	25 ± 2 (n = 23)
Knee-extensor maximum, W	35 ± 2 (n = 16)	19 ± 1 (n = 16)*
Glucose, mg/dl	85 ± 5	99 ± 4*
Total cholesterol, mg/dl	188 ± 12	151 ± 10*
Triglycerides, mg/dl	130 ± 24	131 ± 11
HDL, mg/dl	48 ± 3	38 ± 2*
LDL, mg/dl	123 ± 9	93 ± 7*

HFrEF, heart failure with reduced ejection fraction; HDL, high density lipoprotein; LDL, low density lipoprotein. Data are expressed as means ± SEM. * Significant difference from control, $P < 0.05$.

Table 3.2 *Disease - specific characteristics and medications*

	HFrEF (n = 25)
Disease-specific characteristics	
Left ventricular ejection fraction, % (means ± SEM)	23 ± 2
Diagnosis (ischemic)	16 / 25
Diagnosis (nonischemic)	9 / 25
NYHA class II	18 / 25
NYHA class III	7 / 25
Diabetic	5 / 25
Medications	
β-Blocker	25 / 25
ACE inhibitor	19 / 25
Angiotensin receptor inhibitor	4 / 25
Statin	20 / 25
Diuretic	20 / 25
Aldosterone inhibitor	4 / 25
Calcium channel inhibitor	1 / 25
Digoxin	4 / 25
Anticoagulant	15 / 25
Antiarrhythmic	1 / 25
Erythropoiesis - stimulating agent	1 / 25

HFrEF, heart failure with reduced ejection fraction; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme.

Table 3.3 *Central and peripheral hemodynamics at rest and during handgrip exercise*

Workload (% MVC)	Rest	15%	30%	45%
Control				
Forearm vascular conductance, ml/min/mmHg	1.1 ± 0.2	2.5 ± 0.3†	3.8 ± 0.4†	4.8 ± 0.4†
Brachial artery diameter (cm)	0.47 ± 0.02	0.48 ± 0.02†	0.51 ± 0.02†	0.52 ± 0.02†
Brachial artery blood velocity (cm/sec)	9 ± 1	20 ± 1†	30 ± 2†	37 ± 2†
Heart rate, beats/min	57 ± 2	64 ± 2†	65 ± 2†	68 ± 3†
Stroke volume, ml/beat	109 ± 5	110 ± 5	111 ± 4	110 ± 4
Cardiac output, L/min	6.3 ± 0.3	7.0 ± 0.4†	7.2 ± 0.4†	7.4 ± 0.4†
Systemic vascular conductance, ml/min/mmHg	75 ± 4	77 ± 5	75 ± 4	72 ± 4
Systemic vascular resistance, mmHg/L/min	14 ± 1	14 ± 1	14 ± 1	14 ± 1
HFrEF				
Forearm vascular conductance, ml/min/mmHg	1.1 ± 0.2	2.5 ± 0.2†	3.2 ± 0.3†	3.6 ± 0.3*†
Brachial artery diameter (cm)	0.47 ± 0.02	0.48 ± 0.02	0.48 ± 0.01†	0.49 ± 0.01†
Brachial artery blood velocity (cm/sec)	8 ± 1	21 ± 1†	27 ± 1†	31 ± 1*†
Heart rate, beats/min	67 ± 2*	69 ± 3†	71 ± 2†	73 ± 3†
Stroke volume, ml/beat	83 ± 4*	81 ± 4*	79 ± 4*	76 ± 4*
Cardiac output, L/min	5.4 ± 0.3	5.5 ± 0.2*	5.6 ± 0.3*	5.4 ± 0.3*
Systemic vascular conductance, ml/min/mmHg	68 ± 4	62 ± 3*†	61 ± 4*†	55 ± 3*†
Systemic vascular resistance, mmHg/L/min	17 ± 2	18 ± 2†	20 ± 3*†	21 ± 2*†

MVC, maximum voluntary contraction; HFrEF, heart failure with reduced ejection fraction. Data are expressed as means ± SEM.* Significant difference from control, $P<0.05$; † Significant difference from rest, $P<0.05$.

Table 3.4 *Central and peripheral hemodynamics at rest and during single-leg knee-extensor exercise*

Work rate (W)	Rest	0	5	10	15
Control					
Leg vascular conductance, ml/min/mmHg	3.5 ± 0.3	17.2 ± 0.7†	20.6 ± 0.8†	24.1 ± 0.9†	26.8 ± 1.1†
Common femoral artery diameter, cm	1.05 ± 0.03	1.05 ± 0.03	1.05 ± 0.03	1.05 ± 0.03	1.05 ± 0.03
Common femoral artery blood velocity, cm/sec	6 ± 1	32 ± 2†	34 ± 2†	46 ± 3†	53 ± 3†
Heart rate, beats/min	64 ± 2	72 ± 2†	74 ± 2†	76 ±2†	78 ± 2†
Stroke volume, ml/beat	88 ± 3	92 ± 4	95 ± 5	93 ± 6	97 ± 4
Cardiac output, L/min	5.5 ± 0.2	6.6 ± 0.3†	7.0 ± 0.3†	7.0 ± 0.4†	7.5 ± 0.3†
Systemic vascular conductance, ml/min/mmHg	67 ± 2	71 ± 3	75 ± 4	73 ± 5	75 ± 3
Systemic vascular resistance, mmHg/L/min	15 ± 1	14 ± 1	14 ± 1	15 ± 1	14 ± 1
HFrEF					
Leg vascular conductance, ml/min/mmHg	2.7 ± 0.2	12.6 ± 0.8*†	15.7 ± 0.7*†	17.4 ± 0.8*†	17.6 ± 0.7*†
Common femoral artery diameter, cm	0.96 ± 0.03*	0.96 ± 0.03*	0.96 ± 0.03*	0.96 ± 0.03*	0.96 ± 0.03*
Common femoral artery blood velocity, cm/sec	6 ± 1	30 ± 3†	36 ± 3†	41 ± 4†	43 ± 3†
Heart rate, beats/min	68 ± 2	79 ± 4†	85 ± 5*†	89 ± 5*†	94 ± 4*†
Stroke volume, ml/beat	82 ± 9	92 ± 12	86 ± 11	83 ± 10	82 ± 10
Cardiac output, L/min	5.4 ± 0.5	6.8 ± 0.6†	7.0 ± 0.8†	7.0 ± 0.8†	7.3 ± 0.7†
Systemic vascular conductance, ml/min/mmHg	65 ± 6	70 ± 7	72 ± 8	72 ± 8	69 ± 6
Systemic vascular resistance, mmHg/L/min	19 ± 3	16 ± 2	18 ± 3	17 ± 2	17 ± 2

HFrEF, heart failure with reduced ejection fraction. Data are expressed as means ± SEM.* Significant difference from control, $P<0.05$; † Significant difference from rest, $P<0.05$.

CHAPTER 4

ALPHA-ADRENERGIC RECEPTOR REGULATION OF SKELETAL MUSCLE BLOOD FLOW DURING EXERCISE IN HEART FAILURE WITH REDUCED EJECTION FRACTION

Abstract

Heart failure patients with reduced ejection fraction (HFrEF) experience impaired limb blood flow during exercise, which may be due to disease-related changes in alpha-adrenergic receptor vasoconstriction. Thus, in eight HFrEF patients (63 ± 4 yrs) and eight age- and sex-matched controls (63 ± 4 yrs), we examined changes in leg blood flow (Doppler ultrasound) during intra-arterial infusion of phenylephrine (PE; alpha-1-adrenergic receptor agonist) and phentolamine (PHEN; nonspecific alpha-adrenergic receptor antagonist) at rest and during dynamic single-leg knee-extensor exercise (0, 5, and 10 W). At rest, the reduction in blood flow induced by PE was significantly blunted in HFrEF patients (-15 ± 7%) compared to controls (-36 ± 5%). During exercise, the control subjects exhibited a blunted reduction in blood flow induced by PE (-12 ± 4, -10 ± 4, -9 ± 2% at 0, 5, and 10 W) compared to rest, while the PE-induced change in blood flow was unchanged compared to rest in the HFrEF patients (-8 ± 5, -10 ± 3, -14 ± 3%). PHEN administration increased leg blood flow to a greater extent in HFrEF both at rest (+178 ± 34% vs. +114 ± 28%, HFrEF vs. control) and during exercise (36 ± 6, 37 ± 7, 39 ± 6% vs. 13 ± 3, 14 ± 1, 8 ± 3% at 0, 5, and 10W, HFrEF vs. control). Taken together, these findings implicate disease-related changes in the alpha-adrenergic receptor pathway as an important maladaptive process that restrains exercising skeletal muscle blood flow in HFrEF patients, and may therefore contribute to diminished exercise capacity in this patient group.

Introduction

Heart failure with reduced ejection fraction (HFrEF) is associated with debilitating dyspnea and fatigue triggered by tasks of everyday living, limiting the ability for physical exertion and impairing quality of life (44). Interestingly, while systolic ventricular dysfunction is, by definition, a fundamental characteristic of HFrEF, it does not fully explain the degree of exercise capacity or symptom status in this patient population (8, 20, 27, 46, 49). In contrast, our group (7) and others (57) have documented an attenuated blood flow response to exercising skeletal muscle induced by small muscle mass exercise in HFrEF compared to healthy individuals. Considering the lack of evidence for alternations in perfusion pressure during exercise in patients with HFrEF (30, 39, 44), a blunted rise in vascular conductance is likely the primary contributing factor to the apparent attenuation in exercise hyperemia in this cohort.

A likely culprit offsetting the rise in vascular conductance during exercise in HFrEF is the underlying increase in sympathetic nervous system (SNS) activity in this patient population (13, 25, 48). In an effort to examine the end-organ expression of this exaggerated sympathoexctation in HFrEF, a small number of studies have investigated the effective changes to resting vasomotor tone induced by local intra-arterial infusion of alpha-adrenergic receptor agonists and antagonist drugs. At rest, a qualitatively greater percent increase in forearm blood flow has been documented in response to brachial artery infusion of the nonspecific alpha-adrenergic receptor antagonist phentolamine (PHEN) in patients with HFrEF compared to healthy individuals (57). Additionally, similar dose-dependent reductions in forearm blood flow were observed in response to subsequent administration of phenylephrine (PE) and BHT-993 (selective alpha-1 and alpha-2-adrenegeric receptor agonist drugs, respectively) in the presence of significantly

elevated plasma norepinephrine (NE) (21). Together, these studies indicate a heightened contribution from alpha-adrenergic receptor vasoconstriction to forearm vascular tone in HFrEF patients, coupled with an apparent lack of desensitization of alpha-adrenergic receptors of the forearm vasculature in the presence of chronically heightened sympathoexcitation (21).

These findings at rest raise the question of whether the exaggerated increase in SNS activity during physical activity (28, 30, 39, 42), coupled with the preserved alpha-adrenergic receptor responsiveness (21), could generate a degree of vasoconstriction in the exercising skeletal muscle vasculature that could account for the attenuated exercise hyperemia in this cohort. Using intra-arterial infusion of PHEN to locally abolish sympathetic restraint of skeletal muscle blood flow, Zelis *et al.* (57) documented that alpha-adrenergic receptor-mediated vasoconstriction plays a greater role in restraining blood flow during handgrip exercise in HFrEF patients compared to control subjects, though this response was not evident in a subsequent study using a similar pharmacologic approach during maximal upright cycling (50). However, to our knowledge, the degree to which alpha-adrenergic receptor responsiveness is reduced during exercise in order to optimize blood flow to exercising skeletal muscle, a phenomenon termed "functional sympatholysis" (32), has yet to be determined in patients with HFrEF.

Thus, the present study sought to further determine the role of alpha-1-adrenergic receptor responsiveness, the endogenous contribution alpha-adrenergic receptor pathway to vascular tone and the regulation of skeletal muscle blood flow in leg at rest, as well as determine if these drug-induced changes in vascular tone and blood flow differ as a consequence of exercise of graded intensity in patients with HFrEF and healthy age-

matched controls. We hypothesized that: 1a) At rest, vasoconstriction in response to alpha-1-adrenergic receptor agonist drug infusion (PE) would reduce leg blood flow to a similar degree in HFrEF patients compared to age-matched controls, 1b) At rest, a greater vasodilation in response to alpha-adrenergic antagonist drug infusion (PHEN) would contribute to an enhanced hyperemic response in HFrEF patients compared to age-matched controls, 2a) During exercise, HFrEF patients would exhibit a sustained responsiveness to alpha-1-adrenergic receptor agonist drug infusions into the exercising limb, such that functional sympatholysis would be reduced in HFrEF patients compared to age-matched controls, and 2b) During exercise, HFrEF patients would experience a greater vasodilation to alpha-adrenergic receptor antagonist drug infusion in the exercising limb, such that sympathetic restraint of exercising limb blood flow would be greater in HFrEF patients compared to age-matched controls.

Methods

Subjects

8 New York Heart Association (NYHA) class II-III HFrEF patients and 8 healthy, age- and sex-matched control subjects were recruited either by word of mouth or in the HF clinics at the University of Utah Health Sciences Center and the Salt Lake City VA Medical Center. All age-matched control subjects were nonsmokers, not taking any prescription medication, and were free of overt cardiovascular disease, as indicated by a health history questionnaire. Protocol approval and written informed consent were obtained according to University of Utah and Salt Lake City Veterans Affairs Medical Center Institutional Review Board requirements. All data collection took place at the Utah Vascular Research Laboratory located at the Veterans Affairs Salt Lake City

Geriatric, Research, Education, and Clinical Center. All studies were performed in a thermoneutral environment, with subjects reporting to the laboratory fasted, and not having performed any exercise within 24 hours of the study. Subjects reported to the laboratory on a preliminary day to complete health histories, physical examinations, and perform a graded single-leg knee-extensor (KE) test to determine maximal work rate.

Subjects reported to the UVRL at 0800 on the experimental day. After 20 minutes of supine rest, two catheters (common femoral artery (CFA) and vein (CFV)) were placed using sterile technique, as described previously (2, 5, 6, 56). After catheter placement, subjects rested for ≈30 minutes, and then undertook the protocol as outlined in **Figure 4.1**. Subjects were given a small, standardized meal (1/2 cup of corn flakes and 1/2 cup of skim milk) 5 minutes prior to the start of the exercise portion the alpha-1-adrenergic receptor agonist (PE) trial **(Figure 4.1A)** and the nonspecific alpha-adrenergic receptor antagonist (PHEN) trial **(Figure 4.1B)**. We have previously reported that this meal does not affect leg blood flow at rest or during exercise (6). All data collection took place with subjects in a semirecumbent position (60° reclined). Due to the long lasting effects of PHEN, this portion of the protocol always occurred after the PE portion. Additionally, during both parts of the study, a nonspecific beta-adrenergic receptor antagonist (propranolol) was administered to the healthy control subjects to minimize confounding effects of beta-adrenergic receptor stimulation **(Figure 4.1)**. Propranolol was not administered in the HFrEF patients due to the presence of nonspecific beta-adrenergic receptor antagonists in the patients' respective regimen of daily medication.

Study Drugs

Thigh volumes were determined anthropometrically, and then used for the calculation of drug dosing (23).

Beta-adrenergic receptor antagonist

Propranolol Hydrochloride (APP Pharmaceuticals LLC, Schaumburg, IL) was administered as the nonselective beta-adrenergic receptor antagonist. Propranolol was prepared at a concentration of 0.5 mg/ml in 0.9% sterile saline and 10 ml (5 mg of propranolol) was administered intravenously over the course of 30 seconds before the beginning of both the PE and PHEN protocols **(Figure 4.1)**. This dose has been documented to block beta-adrenergic receptors in the peripheral circulation, ablating the forearm vasodilation response to infusion of epinephrine (Epi) (22).

Alpha-1-adrenergic receptor agonist

Phenylephrine (PE) (Sigma-Aldrich, St. Louis, MO) was administered as a selective alpha-1-adrenergic receptor agonist. PE was prepared at a concentration of 2.5 μg/ml of 0.9% sterile saline. At rest, PE was infused intra-arterially at five doses (0.015, 0.03, 0.06, 0.12, 0.24 μg/min/dl thigh volume, 2 minutes per dose) using a constant-speed infusion pump (Harvard Apparatus, Holliston, MA). During exercise, real-time blood flow was determined prior to PE infusion using ultrasound Doppler, and infusion rate was blood flow adjusted according to these "on-the-fly" blood flow values, to ensure similar effective concentration of the drug at rest and during exercise **(Figure 4.1A)**. This flow-adjusted dose was similar to the 0.12 μg/min/dl thigh volume dose of PE administered at rest, which we have previously reported as sufficient to elicit maximal PE-induced

vasoconstriction, while limiting the risk of systemic spillover during the high infusion rates that occur during single-leg KE exercise (52).

Nonspecific alpha-adrenergic receptor antagonist

Phentolamine Mesylate (PHEN) (Regitine, Bedford Labs, Bedford, OH) was administered as the nonselective alpha-adrenergic receptor antagonist. PHEN was prepared at a concentration of 0.3 mg/ml in 0.9% sterile saline and infused intra-arterially for 10 minutes (total dose: 2.25 mg) **(Figure 4.1B),** followed by a maintenance dose (300 μg/min) for the remainder of the protocol. This dose exceeds that which has been previously documented to achieve complete alpha-adrenergic receptor blockade, as confirmed by alpha-adrenergic receptor agonist challenge (17).

Single-Leg Knee-Extensor Exercise

The single-leg KE paradigm which was implemented in this study has been described previously (3, 4, 6, 24, 33, 55). Briefly, subjects were seated in a semirecumbent position on an adjustable chair with a cycle ergometer (model 828E; Monark Exercise AB, Vansbro, Sweden) positioned behind them. Resistance was created by applying friction to the flywheel, which was turned by the subject via a metal bar connecting the crank arm of the ergometer to a metal boot in which the subject's foot was placed. Subjects exercised for 3 minutes at 0, 5, and 10 W maintaining 60 contractions per minute, with measurements and blood samples taken during the third minute of each stage. During the PE trial **(Figure 4.1A)**, subjects exercised for an additional 2 minutes per stage during which PE was infused, with PE measurements taken during the second minute of infusion. Additionally, the pre-infusion and PE-infusion portions of each work

rate were divided into two separate 3-minute stages if the subject could not continuously exercise for the full 5 minutes. A 5-minute recovery period was given following each stage (**Figure 4.1**), with additional rest given to subjects who required it in order to complete the predetermined stages.

Measurements

Ultrasound Doppler assessments

Measurements of CFA blood velocity and vessel diameter were performed in the infused leg using a Logiq 7 ultrasound Doppler system (GE Medical Systems, Milwaukee, WI) operating in duplex mode. The Logic 7 is equipped with a linear array transducer operating at an imaging frequency of 14 MHz. The CFA was insonated 2-3 cm proximal to the bifurcation of the CFA into the superficial and deep branches. The blood velocity profile was obtained using the same transducer with a Doppler frequency of 5 MHz, operated in the high-pulsed repetition frequency mode (2-25 kHz). Care was taken to avoid aliasing the blood velocity spectra by using scale adjustments, especially during exercise. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less (26). The sample volume was maximized according to vessel size and was centered within the vessel on the basis of real-time ultrasound visualization. At all sample points, arterial diameter (cm) and angle-corrected, time-averaged, and intensity-weighted mean blood velocity (V_{mean}; cm/sec) values were calculated using commercially available software (Logic 7). Using measured arterial diameter and V_{mean}, leg blood flow was calculated according to the equation:

$$\textit{Leg blood flow (ml/min)} = (V_{mean} \times \pi \, (\textit{arterial diameter}/2)^2 \times 60)$$

Blood pressure, vascular conductance, and heart rate assessment

Arterial and venous blood pressure measurements were collected continuously from the indwelling catheters placed in the CFA and CFV with the pressure transducers placed at the level of the catheters (Transpac IV, Abbott Laboratories). Mean arterial pressure (MAP) was calculated as:

$$MAP\ (mmHg) = diastolic\ arterial\ pressure + (arterial\ pulse\ pressure \times 0.33)$$

Leg perfusion pressure was calculated as:

$$Leg\ perfusion\ pressure\ (mmHg) = MAP - venous\ pressure$$

Leg vascular conductance was calculated as:

$$Leg\ vascular\ conductance\ (ml/min/mmHg) = leg\ blood\ flow\ /\ leg\ perfusion\ pressure$$

Heart rate (HR) was monitored from a standard 3-lead ECG recorded in duplicate on the data acquisition device (Biopac, Goleta, CA, U.S.A.) and the Logic 7.

Blood chemistry

A lipid panel was obtained for all subjects by standard techniques. In the last 30 seconds of each exercise stage, femoral arterial and venous blood samples (3-4 ml) were collected. 1 ml of arterial and venous blood were presented anaerobically to a GEM 4000 blood-gas analyzer and co-oximeter (Instrumentation Laboratories, Bedford, MA) to obtain arterial and venous total hemoglobin (tHb) oxyhemoglobin saturation (SO_2), and

partial pressure of oxygen (PO_2), and hematocrit (hct). Arterial and venous blood oxygen (O_2) content (CaO_2 and CvO_2) was calculated as:

$$Blood\ O_2\ content\ (ml/dl) = 1.39\ (tHb) * (SO_2/100) + 0.003 * PO_2$$

Leg O_2 delivery was calculated as:

$$Leg\ O_2\ delivery\ (ml/min) = Leg\ blood\ flow * CaO_2$$

Leg O_2 consumption (VO_2) was calculated as:

$$Leg\ VO_2\ (ml/min) = (CaO_2 - CvO_2) * leg\ blood\ flow$$

The remaining blood was centrifuged, and plasma was stored at -80°C for plasma catecholamine analysis. Plasma NE and Epi concentrations were measured in duplicate by competitive ELISA (2-CAT ELISA; Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany). Using arterial (C_A) and venous (C_V) plasma NE and EPi concentrations, with corrections for leg blood flow, the rate of NE spillover was calculated according to the following equation (37, 38):

$$NE\ spillover\ (ng/min) = [(Cv - Ca) + Ca*(EPi_e)] * (leg\ blood\ flow * ((101 - (hct/100))$$

where EPi_e is the fractional extraction of adrenaline.

Data Analysis

Drug-induced changes were calculated as:

$$\textit{Drug-induced change } (\Delta) = \textit{drug trial value} - \textit{pre-infusion value}$$

or:

$$\textit{Drug-induced change } (\%\Delta) = (\textit{drug-induced change } (\Delta) / \textit{pre-infusion value}) * 100$$

When comparing exercise to resting drug-induced changes for the PE trial, the response to the fourth dose of the PE dose response was always used as this dose was similar to the concentration of drug infused during exercise. The control condition (i.e. pre-infusion) in which the exercise portion of PHEN trial was compared to was the pre-infusion portion of the PE trial **(Figure 4.1)**. Statistics were performed using commercially available software (SigmaStat 3.10; Systat Software, Point Richmond, CA), repeated measures ANOVA ($\alpha < 0.05$), and Student *t-tests* ($\alpha < 0.05$) were used to identify significant changes in measured variables within and between drug groups and across exercise intensities. A Person Product Moment Correlation ($\alpha < 0.05$) was performed to evaluate the association between leg O_2 delivery at 10 W and each subject's respective relative exercise intensity at 10 W of single-leg KE exercise. The Holm-Sidak method was used for alpha adjustment and post hoc analysis. All group data are expressed as means ± SEM.

Results

Subject Characteristics

Baseline characteristics of the control subjects and HFrEF patients are displayed in **Table 4.1**. Disease-specific characteristics and medications of patients with HFrEF are presented in **Table 4.2**.

Beta-Adrenergic Receptor Antagonism

Beta-adrenergic receptor antagonism by intravenous-infusion of propranolol induced a significant reduction in HR, leg blood flow, and leg vascular conductance, with no marked change in leg perfusion pressure or CFA diameter in control subjects **(Table 4.3)**.

Alpha-1-Adrenergic Receptor Responsiveness at Rest

In both the control and HFrEF groups, the administration of the alpha-1-adrenergic receptor agonist PE did not significantly change HR or perfusion pressure with increasing concentrations of the drug **(Table 4.3)**. PE did provoke significant, dose-dependent reductions in leg blood flow, leg vascular conductance, and CFA in both groups **(Figures 4.2, 4.4, and Table 4.3)**. However, compared to the control group, the HFrEF patients exhibited a significantly smaller reduction in leg blood flow and leg vascular **(Figure 4.2 and Table 4.3)** induced by PE infusion.

Nonselective Alpha-Adrenergic Receptor Antagonism at Rest

After 10 minutes of continuous infusion of PHEN, no significant changes in HR or leg perfusion pressure were observed in either group **(Table 4.4)**. Both groups demonstrated a marked increase in leg blood flow and leg vascular conductance in

response to PHEN **(Figures 4.3 and 4.4)**. However, the increases in these variables were significantly greater in HFrEF. Additionally, HFrEF patients exhibited a significantly greater increase in CFA diameter compared to control subjects **(Table 4.4)**.

Cardiovascular Responses to Exercise

Intensity-dependent increases in leg blood flow, leg vascular conductance, and leg perfusion pressure were observed in both groups **(Figures 4.3A and 4.4A)**. However, both the vasodilatory and hyperemic responses were attenuated in HFrEF patients compared to the control group **(Figures 4.3A and 4.4A, top and middle)**. This attenuated hyperemic response across work rates in HFrEF patients contributed to a significantly lower leg O_2 delivery **(Figure 4.6A, top)**. In HFrEF patients, the attenuation in leg O_2 delivery at 10 W was negatively associated with each subject's respective relative exercise intensity at 10 W, an association which was not present in the control group **(Figure 4.7)**. Additionally, the attenuated O_2 delivery and an attenuated leg arterial-venous O_2 difference **(Table 4.5)** contributed to an attenuated leg VO_2 **(Figure 4.6A, bottom)** in HFrEF**.** In both groups, NE spillover increases in an intensity-dependent manner in both groups **(Table 4.5)**. While these values were not significantly different between groups, the HFrEF patients tended to have higher levels of NE spillover compared to control subjects.

Alpha-1-Adrenergic Responsiveness During Exercise

No significant changes in HR or leg perfusion pressure were observed after PE infusion **(Table 4.5).** PE significantly reduced leg blood flow and leg vascular conductance in both groups across increasing exercise intensities **(Figure 4.4A, top and middle)**. Similar PE-induced changes in leg blood flow and leg vascular conductance were also observed in both groups **(Figure 4.4B, top and middle)**. However, PE-induced changes were attenuated compared to rest only in the control group **(Figure 4.4B, top and middle)**. Interestingly, PE only significantly reduced CFA diameter in the HFrEF group during exercise **(Table 4.5)**, and the level of CFA vasoconstriction in HFrEF was not different than the level of vasoconstriction observed at rest across exercise intensities **(Table 4.5)**.

Nonselective Alpha-Adrenergic Antagonism During Exercise

No significant changes in HR were observed between pre-infusion values and PHEN in either group **(Table 4.5)**. PHEN infusion contributed to a reduction in leg perfusion pressure in the control group **(Figure 4.5, bottom)**, while no significant changes in leg perfusion pressure were exhibited in the HFrEF group **(Figure 4.5, bottom)**. PHEN significantly increased leg blood flow and vascular conductance in both groups **(Figure 4.5A, top and middle)**, with the changes in leg blood flow and leg vascular conductance significantly greater in the HFrEF patients compared to control subjects **(Figure 4.5B, top and middle)**. Despite the significant increases in leg blood flow and vascular conductance, these changes were significantly less than the response observed at rest in both groups **(Figure 4.5B, top and middle)**. In the control group, the PHEN-induced increase in leg blood flow, coupled with a reduction in leg arterial-venous

O_2 difference **(Table 4.5),** contributed to similar leg VO_2 values in the PHEN trial compared to pre-infusion values **(Figure 4.6, bottom)**. While the HFrEF patients also exhibited a reduction in leg arterial-venous O_2 difference induced by PHEN **(Table 4.5)**, the PHEN-induced increase in leg blood flow was substantial enough to significantly increase leg VO_2 in this group **(Figure 4.6, bottom)**. Following PHEN infusion, NE spillover was increased in both groups; however, only the control group demonstrated significantly greater values compare to pre-infusion values **(Table 4.5)**.

Discussion

The present study sought to comprehensively examine the importance of alpha-adrenergic receptor-mediated vasoconstriction in the skeletal muscle vasculature of HFrEF patients compared to age-matched control subjects at rest and during exercise. At rest, vasoconstriction in response to intra-arterial infusion of PE was significantly reduced in HFrEF patients, suggesting a disease-related reduction in alpha-1-adrenergic receptor responsiveness. Alpha-adrenergic receptor antagonism induced by intra-arterial infusion of PHEN elicited a greater vasodilation and increase in blood flow in HFrEF patients compared to controls, supporting the concept of exaggerated sympathetic restraint of resting leg blood flow in this patient group. During exercise, PE-induced vasoconstriction and reduction in leg blood flow were reduced compared to rest in the control group. In contrast, vasoconstriction to PE was unchanged from resting responses in HFrEF patients, suggesting that the sympatholytic effect of exercise is impaired in this cohort. Additionally, PHEN infusion during exercise increased leg blood flow to a greater degree in the HFrEF group compared to controls, indicating a greater sympathetic restraint in the leg vasculature during exercise. The PHEN-induced increase in leg blood

flow and the accompanying elevation in O_2 delivery increased leg VO_2 in HFrEF patients, a response not present in the control group. Together, these findings implicate disease-related changes in the alpha-adrenergic receptor pathway as an important maladaptive process that restrains skeletal muscle blood flow and O_2 delivery, and may therefore contribute to diminished exercise capacity and exaggerated exercise intolerance in this patient group.

Alpha-Adrenergic Receptor Regulation at Rest

Though HFrEF patients present with a host of symptoms related to cardiac dysfunction, perhaps one of the most detrimental consequences of the disease is an elevation in SNS activity (25), which has implications in both the progression of HFrEF and mortality of these patients (11). One of the most deleterious effects of heightened sympathoexcitation is the end-organ (i.e. alpha-adrenergic receptor) expression in the peripheral circulation, resulting in a substantial restraint of skeletal muscle blood flow (12). Thus, one of the primary goals of the present study was to determine the role of the alpha-adrenergic receptors in regulating skeletal muscle blood flow at rest in patients with HFrEF.

In the resting leg, administration of PE resulted in significant vasoconstriction in both groups. However, the magnitude of the PE-induced reduction in leg blood flow (**Figure 4.2, top**) and leg vascular conductance (**Figure 4.2, bottom**) were significantly blunted in HFrEF compared with control subjects, suggesting a disease-related reduction in alpha-1 receptor responsiveness in HFrEF patients. These findings are in contrast to one of the only previous human studies to examine alpha-1-mediated vasoconstriction in HFrEF patients. Using multiple doses of PE infused into the brachial artery, Indolfi *et al.*

(18) identified similar reductions in forearm blood flow in NYHA Class II-III HFrEF patients and young, healthy control subjects despite significantly higher concentrations of plasma NE in HFrEF patients. While the reasons for this discrepancy between the former and current studies are not immediately apparent, significant differences in methodology, drug dosing, and subject characteristics may explain the divergent findings observed in the present study. It is also noteworthy that the current study was performed in the leg, an important distinction considering the evidence for nonuniform distribution of alpha-adrenergic receptors between limbs (31). Thus, using a wide range of drug doses that produced a clear "plateau" in vasoconstriction at the highest doses (**Figure 4.2**), we have identified for the first time a reduction in alpha-1-adrenergic receptor-mediated responsiveness in HFpEF patients.

This observed reduction in alpha-1-adrenergic receptor responsiveness is congruent with studies in other populations with elevated SNS activity, where a downregulation and/or desensitization of alpha-adrenergic receptors is observed in response to chronic sympathoexcitation. For example, in the elderly, a population in which SNS activity is also increased (15, 29, 45), our group (54) and others (14, 40) have observed a blunted alpha-adrenergic receptor vasoconstriction following intra-arterial sympathomimetic drug infusions, which likely represents a protective response to the 200-300% increase in resting SNS activity often reported with advancing age. Likewise, it is tempting to speculate that the observed attenuation of the responsiveness of alpha-1-adrenergic receptors in HFrEF patients may be the consequence of a disease-associated increase in SNS activity (19, 25). However, determining whether this disease associated diminution in alpha-1-adrenergic receptor responsiveness is related to a decline in

receptor sensitivity, density, or changes in receptor distribution is beyond the scope of present study.

To further explore disease-related changes in the alpha-adrenergic receptor pathway, we also administered a nonselective alpha-adrenergic receptor antagonist (PHEN) to pharmacologically ablate endogenous alpha-adrenergic receptor tone in the resting leg. In support of our hypothesis, PHEN infusion induced a greater increase in leg blood flow (**Figure 4.3, top**) and leg vascular conductance (**Figure 4.3, bottom**) in HFrEF patients compared to controls. These responses are in agreement with work in a pacing-induced animal model of heart failure (HF), where intra-arterial infusion of PHEN increased resting hindlimb vascular conductance by 50% in healthy control animals and 226% in HF animals (43). Similar observations have been demonstrated in humans. Zelis *et al.* (57) reported a greater percent increase in forearm blood flow in response to brachial artery infusion of PHEN in patients with HFrEF compared to healthy control subjects (57). More recently, Alves *et al.* (1) performed a similar experiment and reported a significantly greater forearm vasodilation and corresponding increase in forearm blood flow in HFrEF patients compared to healthy age-matched control subjects. Thus, the current findings of an exaggerated vasodilation in response to "pharmacologic sympathectomy" in the skeletal muscle vasculature of the leg both confirms and extends previous work in HFrEF patients, supporting the concept that endogenous alpha-adrenergic tone is elevated in this cohort.

Cardiovascular Adjustments to Exercise in HFrEF

It is well-established that patients with HFrEF suffer from an impaired capacity to perform work (44, 51), which may be due, at least in part, to dysfunction in peripheral

hemodynamic regulation. Indeed, work by our group has identified an attenuated hyperemic response during dynamic single-leg KE exercise in this patient group which is related to work capacity (7). The present study extends these recent findings by identifying an impairment in O_2 delivery which is negatively associated with the relative work performed at the highest exercise intensity **(Figure 4.7),** and when coupled with the attenuated leg arterial-venous O_2 difference, contributes to an impairment in leg VO_2 in this patient group **(Figure 4.6)**. This is in agreement with Zelis *et al.* (57), who documented a blunted forearm VO_2 during static-intermittent handgrip exercise in HFrEF patients. However, in this previous study, reduced arm VO_2 was accompanied by a greater arterial-venous O_2 difference in HFrEF patients compared to control subjects, suggestive of a potential limb-specific difference in O_2 extraction during exercise in HFrEF patients. The present findings thus extend this previous work to the dynamic single-leg exercise modality, providing new evidence for a disease-related impairment in the hemodynamic response to small muscle mass exercise that contributes to a reduction in O_2 delivery and VO_2 in this patient group.

Alpha-Adrenergic Receptor Regulation During Exercise

During dynamic exercise, SNS activity increases in effort to redistribute blood flow and optimize perfusion of the exercise skeletal muscle (36). Considering that HFrEF patients experience an exaggerated increase in SNS activity during exercise (18), the alpha-adrenergic receptor pathway provides a logical starting point to probe the attenuated hyperemic response observed in this patient group during exercise. Indeed, inhibition of sympathetic vasoconstriction via the metabolic byproducts produced by the

exercising skeletal muscle, a phenomenon known as "functional sympatholysis" (32), is well-described in both animals (32) and humans (34, 52). However, until now, no studies have examined the sympatholytic effect of exercise in HFrEF.

During exercise, we observed a significant reduction in leg blood flow (**Figure 4.4A, top**) and leg vascular conductance (**Figure 4.4A, middle**) in response to PE administration in both groups across all work rates. However, when compared to the respective responses observed at rest, the control group demonstrated a "lysing" of alpha-1-adrenergic receptor vasoconstriction **(Figure 4.4B, top and middle)**, whereas the HFrEF patients demonstrated a preserved alpha-1-adrenergic receptor vasoconstrictor response **(Figure 4.4B, top and middle)**. This new finding is in agreement with one of the only other studies to examine functional sympatholysis in HF. Using a coronary ligation model of HF in rats, Thomas *et al.* (47) examined changes in femoral artery vascular conductance in response to sympathetic stimulation during both a sham trial and involuntary contraction of the hindlimbs induced by electrical stimulation. The unequivocal finding from this study was that sympathetic vasoconstriction was persevered in these animals during hindlimb contraction compared to the sham trial, indicating an "impaired sympatholysis" in this animal model of HF. The present findings thus extend this former work to the HFrEF patient population, reporting for the first time a preserved alpha-adrenergic receptor vasoconstriction during exercise in this cohort.

To further investigate sympathetic restraint of leg blood flow in HFrEF patients during exercise, alpha-adrenergic receptor antagonism was implemented through intra-arterial infusion of PHEN. Compared to the pre-infusion condition, PHEN administration increased leg blood flow (**Figure 4.5A, top**) and leg vascular conductance (**Figure 4.5A,**

middle) in both groups across all work rates. However, somewhat unexpectedly, leg perfusion pressure was reduced in the control group during the PHEN trial, but remained unchanged in the HFrEF patients (**Figure 4.5, bottom**). Thus, in the control group, this reduction in perfusion pressure during exercise may have contributed to the attenuated increase in leg blood flow, somewhat overestimating the between-group differences in leg blood flow in response to PHEN (**Figure 4.5, top**). However, even when this pressure change was taken into account through calculation of leg vascular conductance (**Figure 4.5, middle**), PHEN-induced changes during exercise remain significantly different between groups (main effect for group), particularly at the higher exercise intensities. Taken together, these data indicate a greater role and capacity of the alpha-adrenergic receptors in restraining blood flow, and ultimately O_2 delivery, in this patient group.

These changes in responses to alpha-adrenergic inhibition are in agreement with animal studies utilizing a similar pharmacologic approach in a pacing-induced HF model. Indeed, Stickland *et al.* (43) observed a substantially greater increase in hindlimb blood flow following PHEN administration during treadmill running in HF animals compared to control animals, indicating an augmented alpha-adrenergic receptor restraint of hindlimb blood flow. Compared to this observation in animals, the results in human HF are equivocal. Zelis *et al.* (57) reported that alpha-adrenergic receptor-mediated vasoconstriction plays a greater role in restraining blood flow during one moderate level of handgrip exercise in HFrEF patients compared to healthy controls, as documented by a larger increase in exercising forearm blood flow in response to intra-arterial infusion of PHEN. In contrast, Wilson *et al.* (50) demonstrated no change in leg blood flow when PHEN was administered prior to maximal upright cycling exercise in HFrEF patients.

The present data build upon this earlier work through inclusion of a carefully matched control group, continuous drug infusion, and use of a the single-leg KE exercise modality at multiple work rates, identifying for the first time a significant sympathetic restraint of leg blood flow during exercise in HFrEF patients compared to controls.

Alpha-Adrenergic Receptor Regulation of Leg Oxygen Consumption During Exercise

While the increase in leg blood flow and leg vascular conductance following PHEN administration clearly identifies the importance of excess sympathetic vasoconstriction on exercise hyperemia in HFrEF, further insight into the functional significance of restricting blood flow may be gained by evaluating leg O_2 delivery and consumption during exercise. Interestingly, after alpha-adrenergic receptor blockade, HFrEF patients demonstrated an increase in leg VO_2, a response not present in the control group **(Figure 4.6, bottom)**. This unique combination of elevated leg VO_2 under the condition of increased O_2 delivery after alpha-adrenergic inhibition suggests a decrease in intramuscular efficiency, defined as the ratio of mechanical output to metabolic cost calculated from VO_2 (10, 41). At face value, a reduction in intramuscular efficiency might appear detrimental, especially in a patient group where being more efficient should be considered beneficial; however, a recent study from our group suggests otherwise. Indeed, using the novel technique of lumbar intrathecal injection of fentanyl to inhibit group III and IV afferent fibers in HFrEF patients during single-leg KE exercise, we observed an increase in leg O_2 delivery and VO_2 in conjunction with a substantial reduction in postexercise quadriceps muscle fatigue. Thus, it is tempting to speculate that what is sacrificed in efficiency when leg blood flow is increased acutely via alpha-

adrenergic receptor blockade (**Figures 4.5 and 4.6**) may be outweighed by reduced fatigue and subsequent improvements in exercise tolerance in HFrEF patients, though additional studies are needed to directly examine this relationship.

Functional Sympatholysis in HFrEF: A Tale of Two Pharmacological Strategies

Conventionally, the concept of functional sympatholysis during exercise is studied using a "stimulatory approach" through the use of lumbar sympathetic stimulation in animal models (47), or through the pharmacological approach of local alpha-adrenergic receptor agonist drugs (9, 16, 53), with both approaches comparing the resting vasoconstrictor response to the response induced during muscular contraction or exercise (**Figure 4.4**). A complimentary approach is the use of alpha-adrenergic receptor antagonism to make comparisons with respect to the change in blood flow at rest and during exercise (**Figure 4.5**). While both of these pharmacological methods probe the role of the alpha-adrenergic receptors in the regulation of vascular tone and blood flow to the exercising skeletal muscle, they should be considered mutually exclusive, as both methods tell very different stories. Specifically, the stimulatory approach is informative regarding the level of vasoconstrictor reserve or "active vasoconstriction" (35) provided by the alpha-adrenergic system. Thus, if sympathetic vasoconstriction during exercise is lysed by metabolic inhibition or alpha-adrenergic receptor occupancy by NE, using a stimulatory approach will indicate to what extent the alpha-adrenergic receptors can further induce vasoconstriction and reduce blood flow. In contrast, alpha-adrenergic antagonism provides insight concerning the level of "passive vasodilation" (35), or the level to which alpha-adrenergic vasoconstriction contributes to the endogenous level of

vascular tone and blood flow both at rest and during exercise. Because of the different aspects of vascular control these two approaches unveil, the current study has comprehensively established the role and vasoconstrictor potential of the alpha-adrenergic receptor pathway at rest and during exercise in this patient group.

Conclusions

This study has revealed a significant role of the alpha-adrenergic receptor pathway in restraining blood flow and O_2 delivery to the exercising skeletal muscle in patients with HFrEF, a physiological maladaptation which might contribute to the evident exercise intolerance and limited capacity to perform tasks of everyday life present in this patient population.

References

1. **Alves MJ, Rondon MU, Santos AC, Dias RG, Barretto AC, Krieger EM, Middlekauff HR, and Negrao CE**. Sympathetic nerve activity restrains reflex vasodilatation in heart failure. *Clin Auton Res* 17: 364-369, 2007.

2. **Amann M, Runnels S, Morgan DE, Trinity JD, Fjeldstad AS, Wray DW, Reese VR, and Richardson RS**. On the contribution of group III and IV muscle afferents to the circulatory response to rhythmic exercise in humans. *J Physiol* 589: 3855-3866, 2011.

3. **Andersen P, Adams RP, Sjogaard G, Thorboe A, and Saltin B**. Dynamic knee extension as model for study of isolated exercising muscle in humans. *J Appl Physiol (1985)* 59: 1647-1653, 1985.

4. **Andersen P, and Saltin B**. Maximal perfusion of skeletal muscle in man. *J Physiol* 366: 233-249, 1985.

5. **Barden J, Lawrenson L, Poole JG, Kim J, Wray DW, Bailey DM, and Richardson RS**. Limitations to vasodilatory capacity and .VO2 max in trained human skeletal muscle. *Am J Physiol Heart Circ Physiol* 292: H2491-2497, 2007.

6. **Barrett-O'Keefe Z, Ives SJ, Trinity JD, Morgan G, Rossman MJ, Donato AJ, Runnels S, Morgan DE, Gmelch BS, Bledsoe AD, Richardson RS, and Wray DW**. Taming the "sleeping giant": the role of endothelin-1 in the regulation of skeletal muscle blood flow and arterial blood pressure during exercise. *Am J Physiol Heart Circ Physiol* 304: H162-169, 2013.

7. **Barrett-O'Keefe Z, Lee JF, Berbert A, Witman MA, Nativi JN, Stehlik J, Richardson RS, and Wray DW**. Hemodynamic Responses to Small Muscle Mass Exercise in Heart Failure Patients with Reduced Ejection Fraction. *Am J Physiol Heart Circ Physiol* 2014.

8. **Benge W, Litchfield RL, and Marcus ML**. Exercise capacity in patients with severe left ventricular dysfunction. *Circulation* 61: 955-959, 1980.

9. **Buckwalter JB, Naik JS, Valic Z, and Clifford PS**. Exercise attenuates alpha-adrenergic-receptor responsiveness in skeletal muscle vasculature. *J Appl Physiol (1985)* 90: 172-178, 2001.

10. **Cavanagh PR, and Kram R**. The efficiency of human movement--a statement of the problem. *Med Sci Sports Exerc* 17: 304-308, 1985.

11. **Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, and Rector T**. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med* 311: 819-823, 1984.

12. **Dargie H**. Sympathetic activity and regional blood flow in heart failure. *Eur Heart J* 11 Suppl A: 39-43, 1990.

13. **Davis D, Baily R, and Zelis R**. Abnormalities in systemic norepinephrine kinetics in human congestive heart failure. *Am J Physiol* 254: E760-766, 1988.

14. **Dinenno FA, Dietz NM, and Joyner MJ**. Aging and forearm postjunctional alpha-adrenergic vasoconstriction in healthy men. *Circulation* 106: 1349-1354, 2002.

15. **Dinenno FA, Jones PP, Seals DR, and Tanaka H**. Limb blood flow and vascular conductance are reduced with age in healthy humans: relation to elevations in sympathetic nerve activity and declines in oxygen demand. *Circulation* 100: 164-170, 1999.

16. **Dinenno FA, Masuki S, and Joyner MJ**. Impaired modulation of sympathetic alpha-adrenergic vasoconstriction in contracting forearm muscle of ageing men. *J Physiol* 567: 311-321, 2005.

17. **Eklund B, and Kaijser L**. Effect of regional alpha- and beta-adrenergic blockade on blood flow in the resting forearm during contralateral isometric handgrip. *J Physiol* 262: 39-50, 1976.

18. **Esposito F, Mathieu-Costello O, Shabetai R, Wagner PD, and Richardson RS**. Limited maximal exercise capacity in patients with chronic heart failure: partitioning the contributors. *J Am Coll Cardiol* 55: 1945-1954, 2010.

19. **Ferguson DW, Berg WJ, and Sanders JS**. Clinical and hemodynamic correlates of sympathetic nerve activity in normal humans and patients with heart failure: evidence from direct microneurographic recordings. *J Am Coll Cardiol* 16: 1125-1134, 1990.

20. **Higginbotham MB, Morris KG, Conn EH, Coleman RE, and Cobb FR**. Determinants of variable exercise performance among patients with severe left ventricular dysfunction. *Am J Cardiol* 51: 52-60, 1983.

21. **Indolfi C, Maione A, Volpe M, Rapacciuolo A, Esposito G, Ceravolo R, Rendina V, Condorelli M, and Chiariello M**. Forearm vascular responsiveness to alpha 1- and alpha 2-adrenoceptor stimulation in patients with congestive heart failure. *Circulation* 90: 17-22, 1994.

22. **Johnsson G, Nyberg G, and Solvell L**. Influence of metoprolol and propranolol on hemodynamic effects induced by physical work and isoprenaline. *Acta Pharmacol Toxicol (Copenh)* 36: 69-75, 1975.

23. **Jones PR, and Pearson J**. Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. *J Physiol* 204: 63P-66P, 1969.

24. **Lawrenson L, Poole JG, Kim J, Brown C, Patel P, and Richardson RS**. Vascular and metabolic response to isolated small muscle mass exercise: effect of age. *Am J Physiol Heart Circ Physiol* 285: H1023-1031, 2003.

25. **Leimbach WN, Jr., Wallin BG, Victor RG, Aylward PE, Sundlof G, and Mark AL**. Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. *Circulation* 73: 913-919, 1986.

26. **Logason K, Barlin T, Jonsson ML, Bostrom A, Hardemark HG, and Karacagil S**. The importance of Doppler angle of insonation on differentiation between 50-69% and 70-99% carotid artery stenosis. *Eur J Vasc Endovasc Surg* 21: 311-313, 2001.

27. **Metra M, Raddino R, Dei Cas L, and Visioli O**. Assessment of peak oxygen consumption, lactate and ventilatory thresholds and correlation with resting and exercise hemodynamic data in chronic congestive heart failure. *Am J Cardiol* 65: 1127-1133, 1990.

28. **Middlekauff HR, Chiu J, Hamilton MA, Fonarow GC, Maclellan WR, Hage A, Moriguchi J, and Patel J**. Muscle mechanoreceptor sensitivity in heart failure. *Am J Physiol Heart Circ Physiol* 287: H1937-1943, 2004.

29. **Ng AV, Callister R, Johnson DG, and Seals DR**. Age and gender influence muscle sympathetic nerve activity at rest in healthy humans. *Hypertension* 21: 498-503, 1993.

30. **Notarius CF, Atchison DJ, and Floras JS**. Impact of heart failure and exercise capacity on sympathetic response to handgrip exercise. *Am J Physiol Heart Circ Physiol* 280: H969-976, 2001.

31. **Pawelczyk JA, and Levine BD**. Heterogeneous responses of human limbs to infused adrenergic agonists: a gravitational effect? *J Appl Physiol (1985)* 92: 2105-2113, 2002.

32. **Remensnyder JP, Mitchell JH, and Sarnoff SJ**. Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake. *Circ Res* 11: 370-380, 1962.

33. **Richardson RS, and Saltin B**. Human muscle blood flow and metabolism studied in the isolated quadriceps muscles. *Med Sci Sports Exerc* 30: 28-33, 1998.

34. **Rosenmeier JB, Dinenno FA, Fritzlar SJ, and Joyner MJ**. alpha1- and alpha2-adrenergic vasoconstriction is blunted in contracting human muscle. *J Physiol* 547: 971-976, 2003.

35. **Rowell LB**. *Human circulation : regulation during physical stress*. New York: Oxford University Press, 1986, p. x, 416 p.

36. **Rowell LB**. Ideas about control of skeletal and cardiac muscle blood flow (1876-2003): cycles of revision and new vision. *J Appl Physiol (1985)* 97: 384-392, 2004.

37. **Savard G, Strange S, Kiens B, Richter EA, Christensen NJ, and Saltin B**. Noradrenaline spillover during exercise in active versus resting skeletal muscle in man. *Acta Physiol Scand* 131: 507-515, 1987.

38. **Savard GK, Richter EA, Strange S, Kiens B, Christensen NJ, and Saltin B**. Norepinephrine spillover from skeletal muscle during exercise in humans: role of muscle mass. *Am J Physiol* 257: H1812-1818, 1989.

39. **Silber DH, Sutliff G, Yang QX, Smith MB, Sinoway LI, and Leuenberger UA**. Altered mechanisms of sympathetic activation during rhythmic forearm exercise in heart failure. *J Appl Physiol* 84: 1551-1559, 1998.

40. **Smith EG, Voyles WF, Kirby BS, Markwald RR, and Dinenno FA**. Ageing and leg postjunctional alpha-adrenergic vasoconstrictor responsiveness in healthy men. *J Physiol* 582: 63-71, 2007.

41. **Stainbsy WN, Gladden LB, Barclay JK, and Wilson BA**. Exercise efficiency: validity of base-line subtractions. *J Appl Physiol Respir Environ Exerc Physiol* 48: 518-522, 1980.

42. **Sterns DA, Ettinger SM, Gray KS, Whisler SK, Mosher TJ, Smith MB, and Sinoway LI**. Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation* 84: 2034-2039, 1991.

43. **Stickland MK, Miller JD, Smith CA, and Dempsey JA**. Carotid chemoreceptor modulation of regional blood flow distribution during exercise in health and chronic heart failure. *Circ Res* 100: 1371-1378, 2007.

44. **Sullivan MJ, and Hawthorne MH**. Exercise intolerance in patients with chronic heart failure. *Prog Cardiovasc Dis* 38: 1-22, 1995.

45. **Sundlof G, and Wallin BG**. Human muscle nerve sympathetic activity at rest. Relationship to blood pressure and age. *J Physiol* 274: 621-637, 1978.

46. **Szlachcic J, Massie BM, Kramer BL, Topic N, and Tubau J**. Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. *Am J Cardiol* 55: 1037-1042, 1985.

47. **Thomas GD, Zhang W, and Victor RG**. Impaired modulation of sympathetic vasoconstriction in contracting skeletal muscle of rats with chronic myocardial infarctions: role of oxidative stress. *Circ Res* 88: 816-823, 2001.

48. **Thomas JA, and Marks BH**. Plasma norepinephrine in congestive heart failure. *Am J Cardiol* 41: 233-243, 1978.

49. **Weber KT, Kinasewitz GT, Janicki JS, and Fishman AP**. Oxygen utilization and ventilation during exercise in patients with chronic cardiac failure. *Circulation* 65: 1213-1223, 1982.

50. **Wilson JR, Ferraro N, and Wiener DH**. Effect of the sympathetic nervous system on limb circulation and metabolism during exercise in patients with heart failure. *Circulation* 72: 72-81, 1985.

51. **Wilson JR, Martin JL, Ferraro N, and Weber KT**. Effect of hydralazine on perfusion and metabolism in the leg during upright bicycle exercise in patients with heart failure. *Circulation* 68: 425-432, 1983.

52. **Wray DW, Fadel PJ, Smith ML, Raven P, and Sander M**. Inhibition of alpha-adrenergic vasoconstriction in exercising human thigh muscles. *J Physiol* 555: 545-563, 2004.

53. **Wray DW, Nishiyama SK, Donato AJ, Sander M, Wagner PD, and Richardson RS**. Endothelin-1-mediated vasoconstriction at rest and during dynamic exercise in healthy humans. *Am J Physiol Heart Circ Physiol* 293: H2550-2556, 2007.

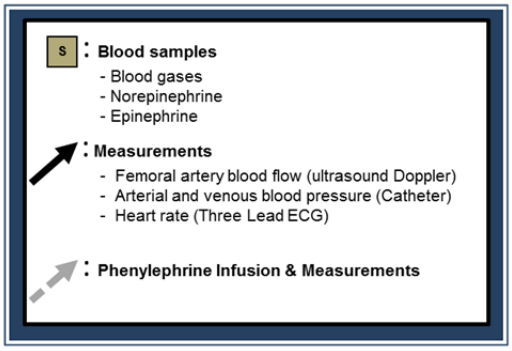
54. **Wray DW, Nishiyama SK, and Richardson RS**. Role of {alpha}1-adrenergic vasoconstriction in the regulation of skeletal muscle blood flow with advancing age. *Am J Physiol Heart Circ Physiol* 296: H497-504, 2009.

55. **Wray DW, Uberoi A, Lawrenson L, and Richardson RS**. Heterogeneous limb vascular responsiveness to shear stimuli during dynamic exercise in humans. *J Appl Physiol (1985)* 99: 81-86, 2005.

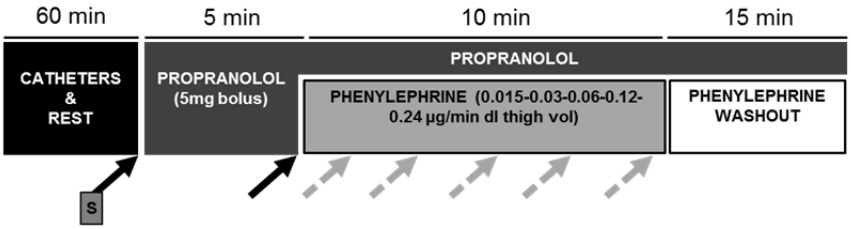
56. **Wray DW, Witman MA, Ives SJ, McDaniel J, Fjeldstad AS, Trinity JD, Conklin JD, Supiano MA, and Richardson RS**. Progressive handgrip exercise: evidence of nitric oxide-dependent vasodilation and blood flow regulation in humans. *Am J Physiol Heart Circ Physiol* 300: H1101-1107, 2011.

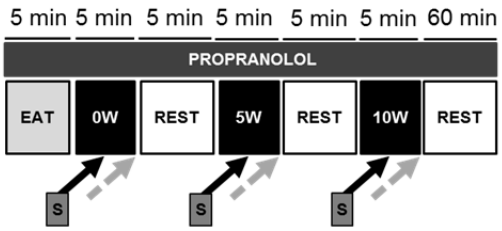
57. **Zelis R, Longhurst J, Capone RJ, and Mason DT**. A comparison of regional blood flow and oxygen utilization during dynamic forearm exercise in normal subjects and patients with congestive heart failure. *Circulation* 50: 137-143, 1974.

Figure 4.1 Experimental timeline for phenylephrine **(A)** and phentolamine **(B)** infusion at rest and during exercise.


S : Blood samples
- Blood gases
- Norepinephrine
- Epinephrine
: Measurements
- Femoral artery blood flow (ultrasound Doppler)
- Arterial and venous blood pressure (Catheter)
- Heart rate (Three Lead ECG)
: Phenylephrine Infusion & Measurements

A


60 min
5 min
10 min
15 min
CATHETERS & REST
PROPRANOLOL (5mg bolus)
PROPRANOLOL
PHENYLEPHRINE (0.015-0.03-0.06-0.12-0.24 µg/min dl thigh vol)
PHENYLEPHRINE WASHOUT
S


5 min 5 min 5 min 5 min 5 min 5 min 60 min
PROPRANOLOL
EAT
0W
REST
5W
REST
10W
REST
S
S
S

B

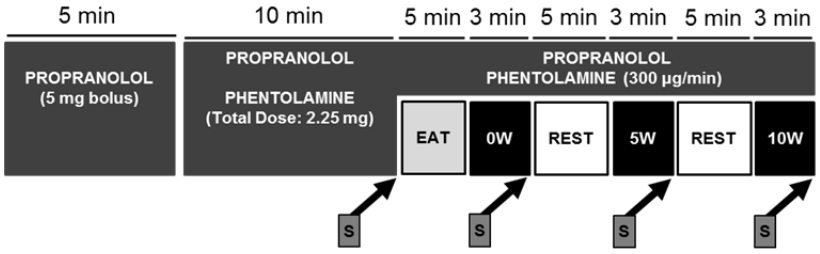

5 min
10 min
5 min 3 min 5 min 3 min 5 min 3 min
PROPRANOLOL (5 mg bolus)
PROPRANOLOL
PHENTOLAMINE (Total Dose: 2.25 mg)
PROPRANOLOL
PHENTOLAMINE (300 µg/min)
EAT
0W
REST
5W
REST
10W
S
S
S
S

Figure 4.2 Changes (%Δ) in leg blood flow (*top*) and leg vascular conductance (*bottom*) in response to local, intra-arterial infusion of phenylephrine (PE) in control subjects and heart failure patients with reduced ejection fraction (HFrEF). ***** Significant difference from control, $P<0.05$; # Significant difference from pre-infusion, $P<0.05$.

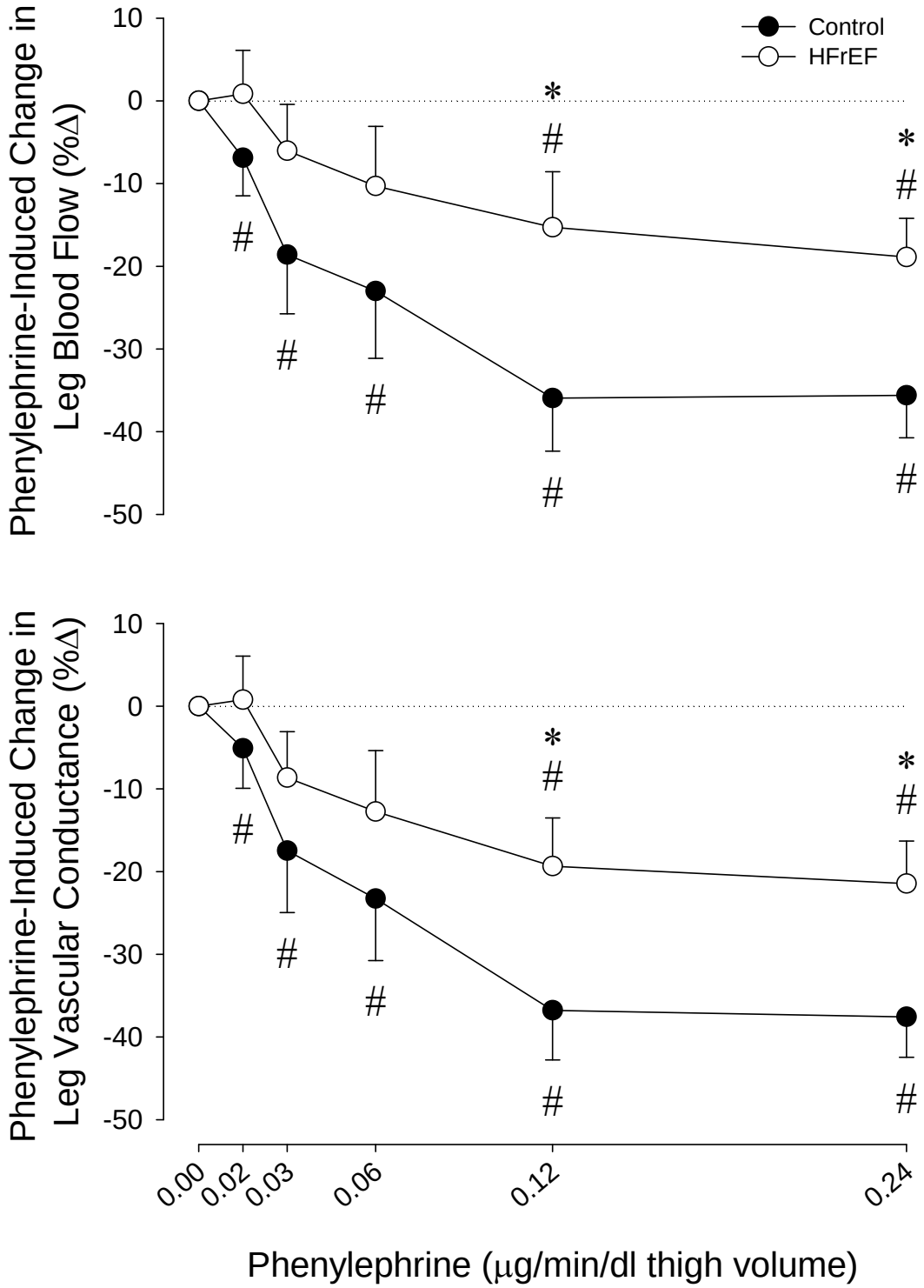

Phenylephrine-Induced Change in Leg Blood Flow (%Δ)
Phenylephrine-Induced Change in Leg Vascular Conductance (%Δ)
Control
HFrEF
10
0
-10
-20
-30
-40
-50
0.00
0.02
0.03
0.06
0.12
0.24
Phenylephrine (μg/min/dl thigh volume)

Figure 4.3 Changes (%Δ) in leg blood flow (*top*) and leg vascular conductance (*bottom*) in response to local, intra-arterial infusion of phentolamine (PHEN) in control subjects and heart failure patients with reduced ejection fraction (HFrEF). ***** Significant difference from control, $P<0.05$; # Significant difference from pre-infusion, $P<0.05$.

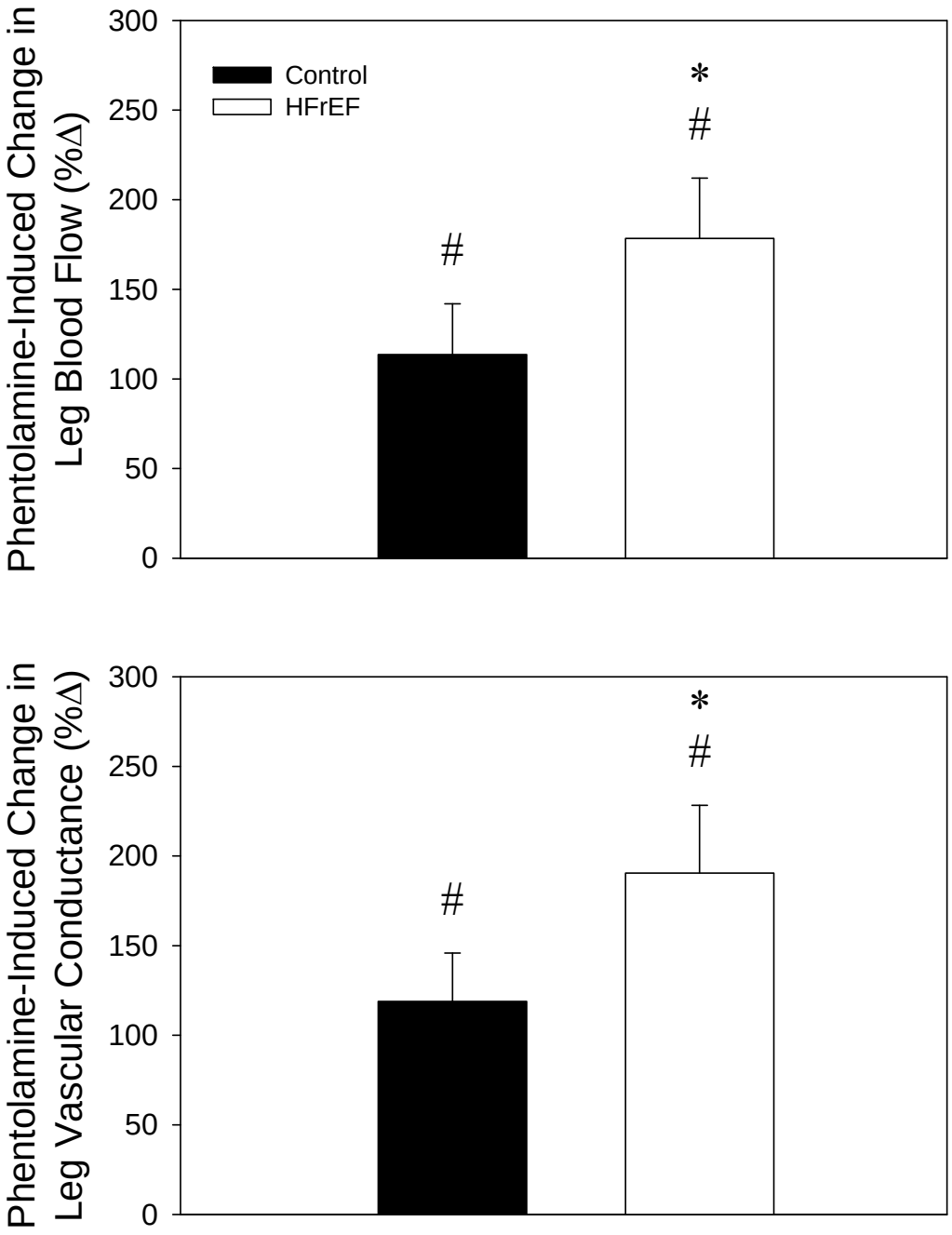

Phentolamine-Induced Change in Leg Blood Flow (%Δ)
300
250
200
150
100
50
0
Control
HFrEF
*
#
#
Phentolamine-Induced Change in Leg Vascular Conductance (%Δ)
300
250
200
150
100
50
0
*
#
#

Figure 4.4 Absolute values **(A)** of: Leg blood flow (*top*), leg vascular conductance (*middle*), and leg perfusion pressure (*bottom*) in control subjects and heart failure patients with reduced ejection fraction (HFrEF) at rest and during knee-extensor exercise in control conditions (pre-infusion) and during phenylephrine (PE) infusion. PE-induced changes (%Δ) **(B)** of: leg blood flow (*top*), leg vascular conductance (*middle*), and leg perfusion pressure (*bottom*) in control subjects and HFrEF patients at rest and during knee-extensor exercise. ***** Significant difference from control, $P<0.05$; # Significant difference from pre-infusion, $P<0.05$; † Significant difference from rest, $P<0.05$.

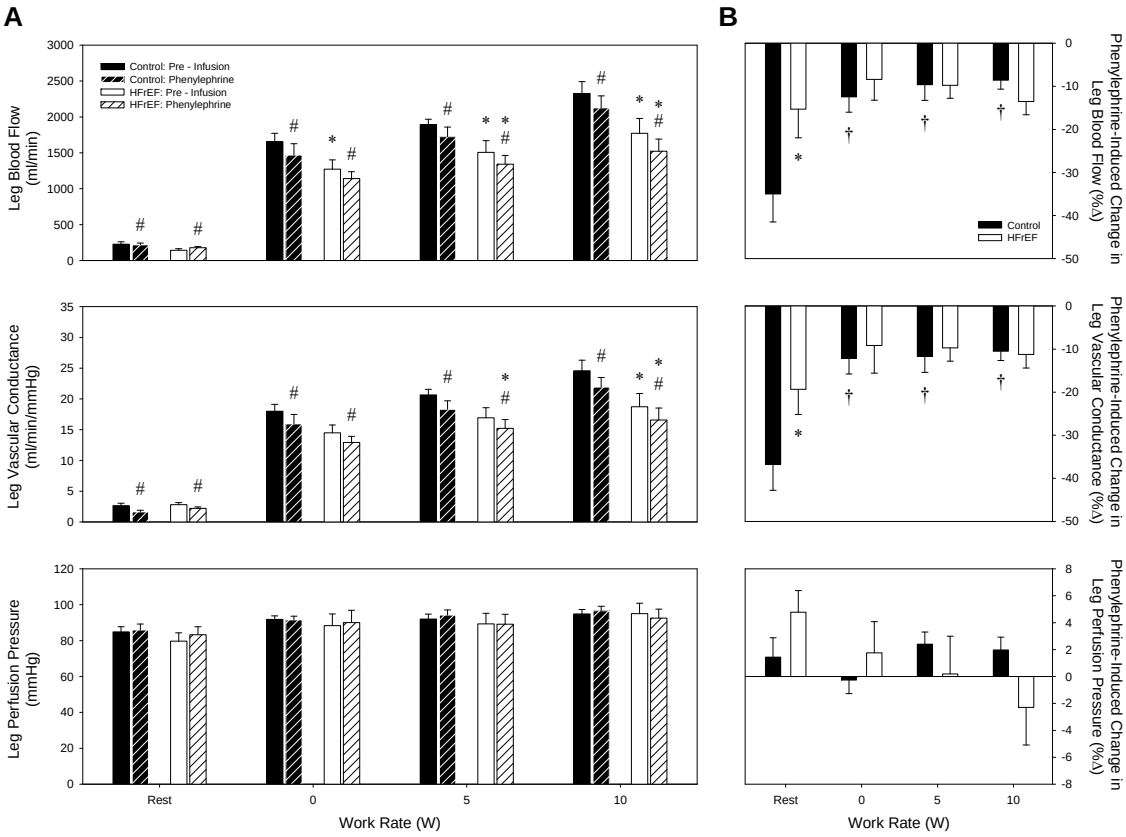

A
B
Control: Pre - Infusion
Control: Phenylephrine
HFrEF: Pre - Infusion
HFrEF: Phenylephrine
Leg Blood Flow (ml/min)
Leg Vascular Conductance (ml/min/mmHg)
Leg Perfusion Pressure (mmHg)
Phenylephrine-Induced Change in Leg Blood Flow (%Δ)
Phenylephrine-Induced Change in Leg Vascular Conductance (%Δ)
Phenylephrine-Induced Change in Leg Perfusion Pressure (%Δ)
Control
HFrEF
Rest
0
5
10
Work Rate (W)

Figure 4.5 Absolute values **(A)** of: Leg blood flow (*top*), leg vascular conductance (*middle*), and leg perfusion pressure (*bottom*) in control subjects and heart failure patients with reduced ejection fraction (HFrEF) at rest and during knee-extensor exercise in control conditions (pre-infusion) and during phentolamine (PHEN) infusion. PHEN-induced changes (%Δ) **(B)** of: Leg blood flow (*top*), leg vascular conductance (*middle*), and leg perfusion pressure (*bottom*) in control subjects and HFrEF patients at rest and during knee-extensor exercise. * Significant difference from control, $P<0.05$; # Significant difference from pre-infusion, $P<0.05$; † Significant difference from rest, $P<0.05$.

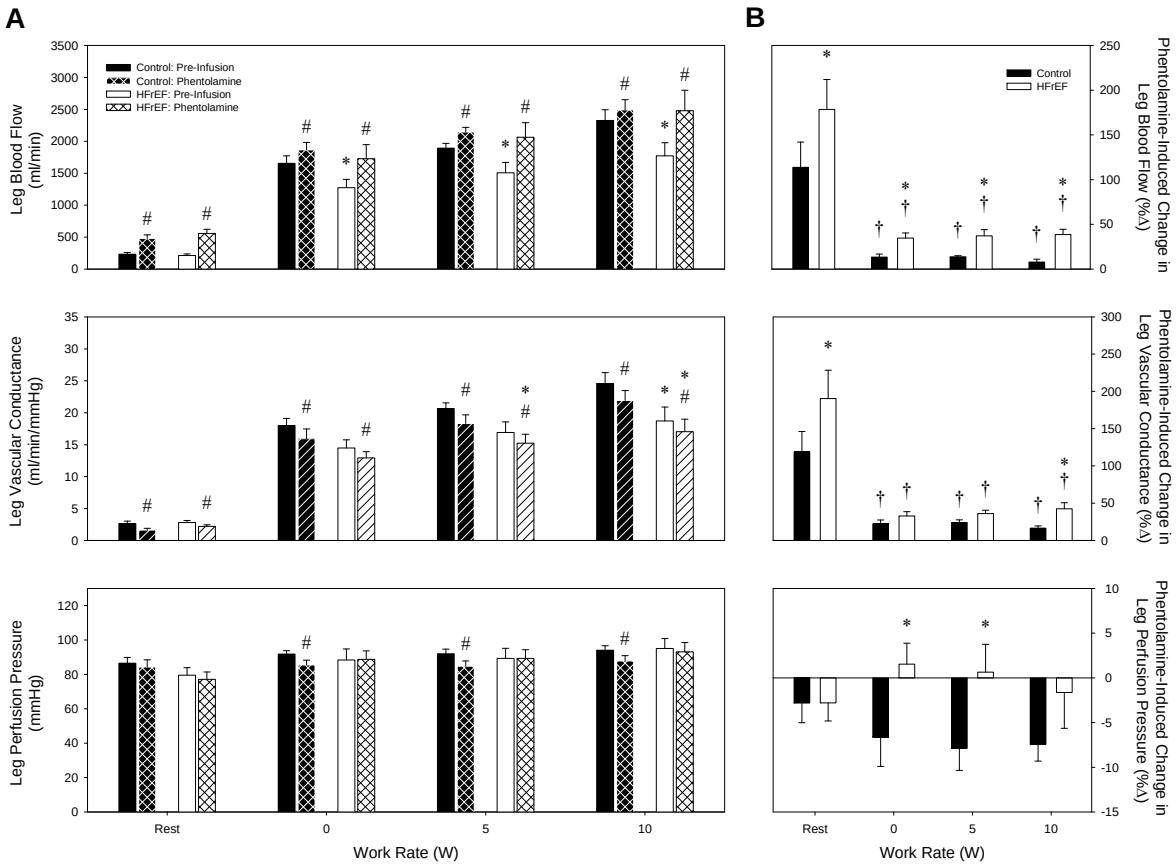

A
B
Control: Pre-Infusion
Control: Phentolamine
HFrEF: Pre-Infusion
HFrEF: Phentolamine
Leg Blood Flow (ml/min)
Leg Vascular Conductance (ml/min/mmHg)
Leg Perfusion Pressure (mmHg)
Phentolamine-Induced Change in Leg Blood Flow (%Δ)
Phentolamine-Induced Change in Leg Vascular Conductance (%Δ)
Phentolamine-Induced Change in Leg Perfusion Pressure (%Δ)
Control
HFrEF
Rest
0
5
10
Work Rate (W)

Figure 4.6 Absolute values **(A)** of: Leg oxygen delivery (*top*), and leg oxygen consumption (*bottom*) in control subjects and heart failure patients with reduced ejection fraction (HFrEF) at rest and during knee-extensor exercise in control conditions (pre-infusion) and during phentolamine (PHEN) infusion. PHEN-induced changes (Δ) **(B)** of: Leg oxygen delivery (*top*) and leg oxygen consumption (*bottom*) in control subjects and HFrEF patients at rest and during knee-extensor exercise. * Significant difference from control, $P<0.05$; # Significant difference from pre-infusion, $P<0.05$; † Significant difference from rest, $P<0.05$.

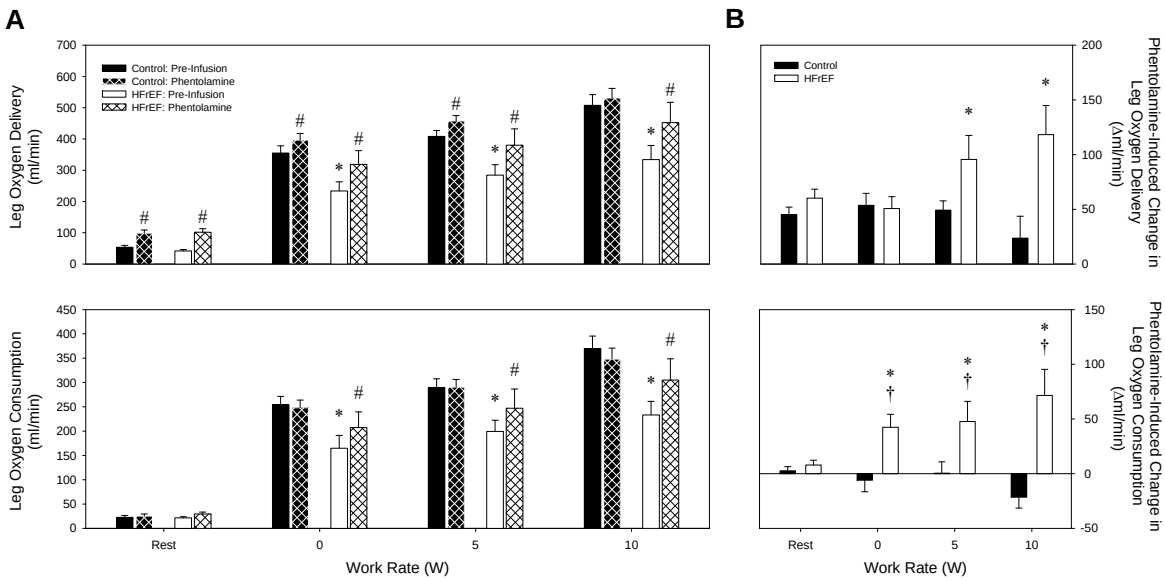

A
B
Control: Pre-Infusion
Control: Phentolamine
HFrEF: Pre-Infusion
HFrEF: Phentolamine
Leg Oxygen Delivery (ml/min)
Leg Oxygen Consumption (ml/min)
Control
HFrEF
Phentolamine-Induced Change in Leg Oxygen Delivery (Δml/min)
Phentolamine-Induced Change in Leg Oxygen Consumption (Δml/min)
Rest
0
5
10
Work Rate (W)

Figure 4.7 Relationship between leg oxygen delivery at 10 W and relative intensity at 10 W during the control condition (pre-infusion) in control subjects and heart failure patients with reduced ejection fraction (HFrEF). Significant Pearson Product Moment Correlation coefficient, $P<0.05$.

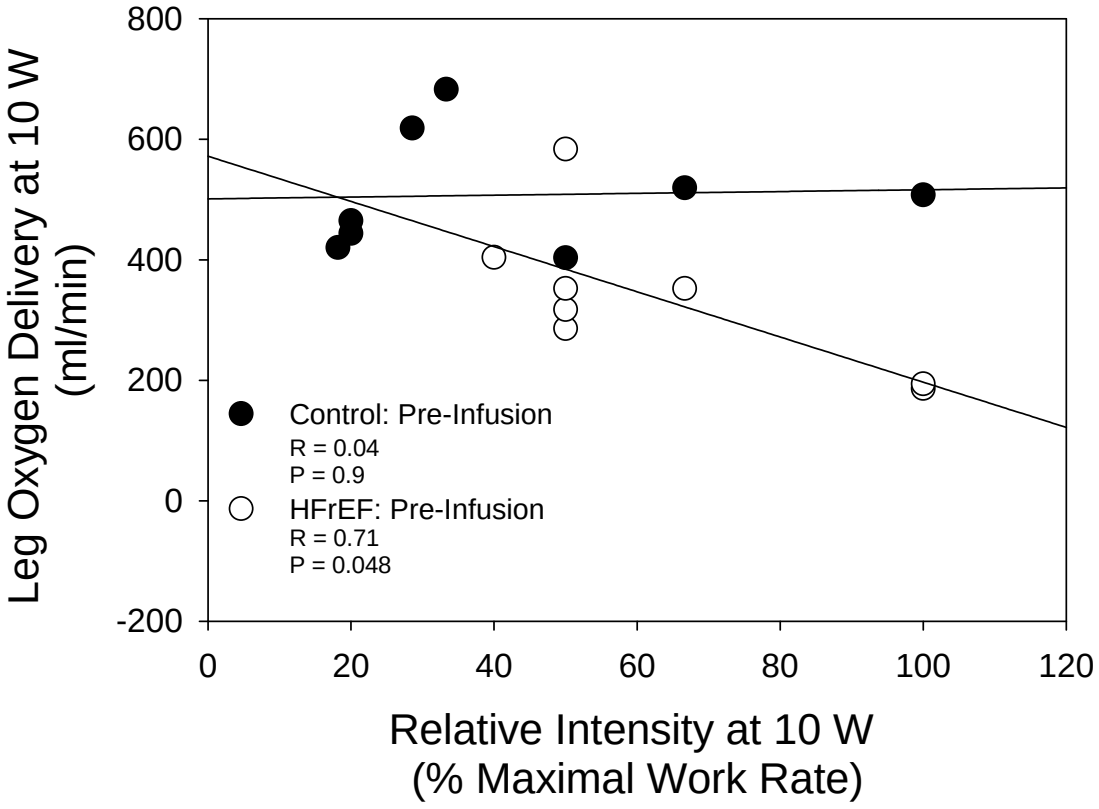

800
600
400
200
0
-200
0
20
40
60
80
100
120
Leg Oxygen Delivery at 10 W
(ml/min)
Relative Intensity at 10 W
(% Maximal Work Rate)
Control: Pre-Infusion
R = 0.04
P = 0.9
HFrEF: Pre-Infusion
R = 0.71
P = 0.048

Table 4.1 *Subject characteristics*

	Control (n = 8)	HFrEF (n = 8)
Age, yrs	63 ± 4	63 ± 4
Height, cm	174 ± 3	174 ± 3
Weight, kg	76 ± 6	82 ± 4
Body mass index, kg/m^2	25 ± 1	27 ± 1
Thigh volume, L	7.7 ± 0.5	5.9 ± 0.1*
Quadriceps muscle mass, kg	2.7 ± 0.1	2.2 ± 0.0*
Systolic blood pressure, mmHg	120 ± 3	120 ± 6
Diastolic blood pressure, mmHg	79 ± 2	77 ± 3
Knee-extensor maximum, W	33 ± 6	18 ± 2*
Glucose, mg/dl	86 ± 4	99 ± 8
Total cholesterol, mg/dl	195 ± 14	139 ± 19*
Triglycerides, mg/dl	117 ± 24	103 ± 10
HDL, mg/dl	47 ± 4	37 ± 3
LDL, mg/dl	131 ± 13	86 ± 15*

HFrEF, heart failure with reduced ejection fraction; HDL, high density lipoprotein; LDL, low density lipoprotein. Data are expressed as means ± SEM. * Significant difference from control, $P < 0.05$.

Table 4.2 *Disease - specific characteristics and medications*

	HFrEF (n = 8)
Disease-specific characteristics	
Left ventricular ejection fraction, % (means ± SEM)	30 ± 3
Diagnosis (ischemic)	6 / 8
Diagnosis (nonischemic)	2 / 8
NYHA class II	4 / 8
NYHA class III	4 / 8
Diabetic	4 / 8
Medications	
β-Blocker	8 / 8
ACE inhibitor	6 / 8
Angiotensin receptor inhibitor	2 / 8
Statin	7 / 8
Diuretic	8 / 8
Aldosterone inhibitor	2 / 8
Digoxin	1 / 8
Anticoagulant	7 / 8

HFrEF, heart failure with reduced ejection fraction; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme.

Table 4.3 *Hemodynamic responses to propranolol and phenylephrine at rest*

			Phenylephrine (ug/min/dl thigh volume)				
	Baseline	Propranolol	0.015	0.03	0.06	0.12	0.24
Control							
Leg blood flow, ml/min	253 ± 33	227 ± 33#	212 ± 33	185 ± 33#	174 ± 33#	142 ± 24#	141 ± 19#
Leg vascular conductance , ml/min/mmHg	3.0 ± 0.4	2.7 ± 0.4#	2.5 ± 0.4	2.2 ± 0.4#	2.1 ± 0.4#	1.6 ± 0.3#	1.6 ± 0.2#
Leg perfusion pressure, mmHg	85 ± 3	85 ± 3	83 ± 3	84 ± 3	85 ± 3	86 ± 3	87 ± 3
Common femoral artery diameter, cm	1.02 ± 0.06	1.02 ± 0.06	1.01 ± 0.06	0.99 ± 0.06#	0.97 ± 0.07#	0.97 ± 0.07#	0.93 ± 0.7#
Common femoral artery diameter, %Δ	-	0.0 ± 0.1	-0.6 ± 0.4	-3.0 ± 1.7	-4.8 ± 2.5#	-6.8 ± 2.9#	-9.6 ± 3.5#
Heart rate, bpm	60 ± 4	54 ± 3#	54 ± 2	53 ± 3	53 ± 2	51 ± 2	52 ± 2
HFrEF							
Leg blood flow, ml/min	218 ± 26	-	215 ± 20	202 ± 22	189 ± 19	179 ± 16#	174 ± 18#
Leg vascular conductance, ml/min/mmHg	2.8 ± 0.3	-	2.8 ± 0.3	2.5 ± 0.3	2.3 ± 0.2#	2.2 ± 0.3#	2.1 ± 0.2#
Leg perfusion pressure, mmHg	80 ± 5	-	80 ± 5	82 ± 5	82 ± 5	83 ± 4	82 ± 3
Common femoral artery diameter, cm	0.88 ± 0.05	-	0.87 ± 0.05	0.86 ± 0.05	0.84 ± 0.05#	0.82 ± 0.05#	0.80 ± 0.06#
Common femoral artery diameter, %Δ	-	-	-1.2 ± 0.3	-2.6 ± 0.6	-4.3 ± 0.8#	-6.2 ± 1.0#	-8.8 ± 2#
Heart rate, bpm	67 ± 2	-	65 ± 2*	66 ± 3*	67 ± 2*	68 ± 3*	72 ± 6*

Data are expressed as means ± SEM.* Significant difference from control, P<0.05; # Significant difference from pre-infusion value.

Table 4.4 *Hemodynamic responses to infusions of propranolol and phentolamine at rest*

	Baseline	Propranolol	Phentolamine
Control			
Leg blood flow, ml/min	234 ± 26	230 ± 27	477 ± 60#
Leg vascular conductance, ml/min/mmHg	2.7 ± 0.3	2.7 ± 0.3	5.7 ± 0.6#
Leg perfusion pressure, mmHg	87 ± 3	86 ± 4	84 ± 4
Common femoral artery diameter, cm	1.02 ± 0.06	1.03 ± 0.05	1.05 ± 0.05
Common femoral artery diameter, %Δ	-	1.1 ± 1.1	1.7 ± 1.2
Heart rate, bpm	56 ± 3	54 ± 2	58 ± 3
HFrEF			
Leg blood flow, ml/min	212 ± 24	-	557 ± 66#
Leg vascular conductance, ml/min/mmHg	2.8 ± 0.3	-	7.4 ± 1.0*#
Leg perfusion pressure, mmHg	79 ± 4	-	77 ± 4
Common femoral artery diameter, cm	0.88 ± 0.05	-	0.91 ± 0.06#
Common femoral artery diameter, %Δ	-	-	4.1 ± 0.9#
Heart rate, bpm	67 ± 3*	-	67 ± 3*

Data are expressed as means ± SEM.* Significant difference from control, $P<0.05$;
Significant difference from pre-infusion value, $P<0.05$.

Table 4.5 *Cardiovascular responses to intra-arterial infusions of phenylephrine and phentolamine at rest and during exercise*

	Rest	0 W	5 W	10 W
PRE-INFUSION				
Control				
Heart rate, bpm	55 ± 2	66 ± 3†	70 ± 5†	72 ± 5†
Leg arterial-venous O_2 difference, ml/dl	9.3 ± 1.3	15.4 ± 0.6†	15.4 ± 0.8†	16.0 ± 0.6†
Norepinephrine spillover, ng/min	71 ± 16	384 ± 54†	417 ± 48†	426 ± 75†
Common femoral artery diameter, cm	1.02 ± 0.05	1.02 ± 0.05	1.02 ± 0.05	1.02 ± 0.05
HFrEF				
Heart rate, bpm	65 ± 3*	84 ± 5*†	86 ± 2*†	92 ± 3*†
Leg arterial-venous O_2 difference, ml/dl *	10.1 ± 0.6	12.7 ± 1.1†	13.4 ± 0.8†	13.4 ± 0.8†
Norepinephrine spillover, ng/min	79 ± 22†	418 ± 163†	598 ± 248†	689 ± 144†
Common femoral artery diameter, cm	0.88 ± 0.05	0.88 ± 0.05	0.88 ± 0.05	0.88 ± 0.05
PHENYLEPHRINE				
Control				
Heart rate, bpm	-	65 ± 4†	68 ± 6†	71 ± 5†
Common femoral artery diameter, cm	0.95 ± 0.07#	1.00 ± 0.06†	1.00 ± 0.05†	1.01 ± 0.05†
Common femoral artery diameter, %Δ	-6.8 ± 2.9	-2.2 ± 0.7†	-2.2 ± 0.6†	-1.1 ± 0.5†
HFrEF				
Heart rate, bpm	-	81 ± 5*†	82 ± 4*†	89 ± 2*†
Common femoral artery diameter, cm	0.82 ± 0.05	0.83 ± 0.05*	0.83 ± 0.05*	0.83 ± 0.05
Common femoral artery diameter, %Δ	-6.2 ± 1.0#	-5.3 ± 1.2#	-4.1 ± 1.3#	-2.5 ± 1.0
PHENTOLAMINE				
Control				
Heart rate, bpm	58 ± 3	68 ± 5†	74 ± 7†	75 ±6†
Leg arterial-venous O_2 difference, ml/dl	6.4 ± 1.6#	13.5 ± 0.8#†	13.6 ± 0.8#†	14.1 ± 0.7#†
Norepinephrine spillover, ng/min	218 ± 28	982 ± 259#†	1105 ± 334#†	1030 ± 195#†
Common femoral artery diameter, cm	1.05 ± 0.05	1.05 ± 0.05	1.05 ± 0.05	1.05 ± 0.05
HFrEF				
Heart rate, bpm	67 ± 3*	83 ± 6†	88 ± 5†	97 ± 6*†
Leg arterial-venous O_2 difference, ml/dl #	5.6 ± 0.6	12.0 ± 0.7†	11.9 ± 0.9†	12.3 ± 0.6†
Norepinephrine spillover, ng/min	221 ± 42	774 ± 230†	669 ± 206†	1062 ± 292†
Common femoral artery diameter, cm	0.91 ± 0.06#	0.91 ± 0.06#	0.91 ± 0.06#	0.91 ± 0.06#

Data are expressed as means ± SEM. O_2, oxygen.* Significant difference from control, $P<0.05$; # Significant difference from pre-infusion, $P<0.05$; † Significant difference from rest, $P<0.05$.

CHAPTER 5

CONCLUSION

A hallmark characteristic of patients with HFrEF is a heightened sympathetic nervous system (SNS) activity, quantified by resting muscle sympathetic nerve activity (MSNA) (2, 4) and plasma norepinephrine concentration (3), both of which are associated with severity (2, 3) and predictive of mortality in HFrEF patients (1, 3). One of the principal functional consequences of the exaggerated SNS activation and subsequent peripheral vasoconstriction in patients with HFrEF might be the worsening of symptoms upon exertion. Indeed, previous studies exploring the possible relation between exercise intolerance in HFrEF and SNS activity indicated that resting MSNA is negatively associated with aerobic capacity in this cohort (6), and patients with HFrEF exhibit an exaggerated increase in MSNA during exercise (5, 7-9). These novel findings raise the question of the functional end-organ consequences of the persistent augmentation of MSNA during exercise in HFrEF. Thus, the purpose of the studies encompassing this dissertation was to systematically investigate the hemodynamic response to exercise in HFrEF and healthy controls of a similar age, with an emphasis on how SNS overactivity may contribute to dysregulation of the cardiovascular system in this cohort.

In the first study, we aimed to determine how varying levels of metaboreceptor activation alter the mean arterial pressure (MAP) response, as well as the degree to which cardiac output (CO) and systemic vascular conductance (SVC) contribute to the metaboreflex-induced pressor response. Across workloads, the metaboreflex-induced increase in MAP was similar between groups. In controls, this was driven by increases in CO; however, in HFrEF, this change was accomplished by reductions in SVC, which contributed to an exaggerated increase in effective arterial elastance which was associated with an attenuated increase in stroke work in the patient group. Together, these

findings indicate a preserved role of the metaboreflex-induced pressor response in HFrEF, but suggest that this response is governed by the peripheral circulation, a maladaptation that may exacerbate existing systolic dysfunction in this cohort.

The objective of the second study was to evaluate the hemodynamic responses to small muscle mass exercise in HFrEF patients and healthy control subjects of a similar age. During HG exercise, at 15% maximum voluntary contraction (MVC), forearm blood flow was similar between groups, while HFrEF patients exhibited an attenuated increase at the two highest intensities compared to control subjects, with the greatest difference at the highest workload. During knee-extensor exercise, HFrEF patients exhibited a diminished leg hyperemic response across all work rates. Together, these findings indicate a marked attenuation in exercising limb perfusion attributable to impairments in peripheral vasodilatory capacity during both arm and leg exercise in patients with HFrEF, which likely plays a role in limiting exercise capacity in this patient population.

The objective of the third study was to investigate what role the alpha-adrenergic receptors play in restraining leg blood flow to the exercising limb in patients with HFrEF and age-matched control subjects. At rest, HFrEF patients exhibited an attenuated vasoconstrictor response to the local intra-arterial infusion of the alpha-1 adrenergic receptor agonist phenylephrine (PE). During exercise, the responsiveness to PE was blunted compared to rest in control subjects, but preserved in HFrEF patients. Additionally, HFrEF patients exhibited an enhanced alpha-adrenergic receptor restraint of limb blood flow, as demonstrated by the enhanced hyperemic response induced by the nonspecific alpha-adrenergic receptor antagonist phentolamine (PHEN) both at rest and during exercise. Together, this study indicates that alpha-adrenergic receptor restraint of

leg blood flow is upregulated at rest during exercise in patients with HFrEF and potentially contributes to the impaired exercise capacity exhibited to this patient group.

It is of utmost importance that we understand and study the cardiovascular response to exercise in patients with HFrEF in order to investigate therapeutic avenues to improve symptom status in this patient population during exercise. Findings from the studies contained herein may help to achieve this goal, by demonstrating the cardiovascular maladaptations associated with this disease and the role the SNS plays. We propose that the findings regarding the SNS demonstrate the potential for further targeting of this pathway in the treatment of HFrEF.

References

1. **Barretto AC, Santos AC, Munhoz R, Rondon MU, Franco FG, Trombetta IC, Roveda F, de Matos LN, Braga AM, Middlekauff HR, and Negrao CE**. Increased muscle sympathetic nerve activity predicts mortality in heart failure patients. *Int J Cardiol* 135: 302-307, 2009.

2. **Ferguson DW, Berg WJ, and Sanders JS**. Clinical and hemodynamic correlates of sympathetic nerve activity in normal humans and patients with heart failure: evidence from direct microneurographic recordings. *J Am Coll Cardiol* 16: 1125-1134, 1990.

3. **Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH, Rudin-Toretsky E, and Yusuf S**. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). *Circulation* 82: 1724-1729, 1990.

4. **Leimbach WN, Jr., Wallin BG, Victor RG, Aylward PE, Sundlof G, and Mark AL**. Direct evidence from intraneural recordings for increased central sympathetic outflow in patients with heart failure. *Circulation* 73: 913-919, 1986.

5. **Middlekauff HR, Chiu J, Hamilton MA, Fonarow GC, Maclellan WR, Hage A, Moriguchi J, and Patel J**. Muscle mechanoreceptor sensitivity in heart failure. *Am J Physiol Heart Circ Physiol* 287: H1937-1943, 2004.

6. **Notarius CF, Ando S, Rongen GA, and Floras JS**. Resting muscle sympathetic nerve activity and peak oxygen uptake in heart failure and normal subjects. *Eur Heart J* 20: 880-887, 1999.

7. **Notarius CF, Atchison DJ, and Floras JS**. Impact of heart failure and exercise capacity on sympathetic response to handgrip exercise. *Am J Physiol Heart Circ Physiol* 280: H969-976, 2001.

8. **Silber DH, Sutliff G, Yang QX, Smith MB, Sinoway LI, and Leuenberger UA**. Altered mechanisms of sympathetic activation during rhythmic forearm exercise in heart failure. *J Appl Physiol* 84: 1551-1559, 1998.

9. **Sterns DA, Ettinger SM, Gray KS, Whisler SK, Mosher TJ, Smith MB, and Sinoway LI**. Skeletal muscle metaboreceptor exercise responses are attenuated in heart failure. *Circulation* 84: 2034-2039, 1991.